

Supplement 2. Questionnaire of the study Prevalence and risk factors for the occurrence of ESBL *E. coli* in pre-weaned dairy calves and their dams on large dairy farms in Germany - a cross-sectional study

Data of the participants

General Data

- ☐ conventional farming
- ☐ organic farming (since _____)

■ Are the other livestock husbandries within a radius of 3 km?

- ☐ yes, _____ (species)
- ☐ no

■ Frequently buy-in of cattle?

- ☐ yes ☐ no

If yes, how often?

- ☐ more than once a month
- ☐ every 1-3 months
- ☐ less than every 3 months

Information of the origin farm/s

- ☐ only 1 origin farm
- ☐ > 1 fixed origin farms
- ☐ > 1 changing farms

■ Outsourced heifer rearing?

- ☐ yes ☐ no

If yes, in

- ☐ another location of the own farm
- ☐ an external farm

☐ heifers are raised at more than 1 location _____ (number)

☐ there are "foreign" animals at this location

■ Which animals feeds are used for calves and cows:

☐ in-house basic forage _____

☐ in-house substrates _____

☐ external basic forage _____

☐ external substrates _____

☐ bought in forage comes from a single farm

☐ bought in forage comes from different farms

How often do the producers change?

☐ > 1 x / month

☐ every 1-3 months

☐ less than every 3 months

■ Fertilization of the fields that are used for the feed/bedding material production

☐ in-house slurry

☐ external slurry

■ Does an in-house biogas reactor exist?

☐ yes ☐ no

If yes, do you use in-house material exclusively?

☐ yes ☐ no, _____

Are the fields that are used for feed production or bedding material fertilized with the outcome of the biogas plant, that were fed with foreign slurry/manure?

☐ yes ☐ no

How many foreign husbandries are involved?

☐ 1-3

☐ more than 3

How often do foreign husbandries change?

- ☐ never
- ☐ more than every 3 months
- ☐ less than every 3 months

Which foreign husbandries are we talking about? (species)

- ☐ _____
- ☐ _____
- ☐ _____

■ Which people have contact with the livestock animals?

- ☐ personnel
- ☐ veterinarian
- ☐ family members
- ☐ visitors (e. g. providers, craftsman)

Do this people have contact to other animals?

- ☐ yes, to _____ (species)
- ☐ no

■ Do you perform pest control?

- ☐ yes, _____
- ☐ no

Calving management

- Housing:
- ☐ single pen
 - ☐ group pen
 - ☐ straw bedding
 - ☐ _____

Hygiene-management:

Cleaning frequency?

- ☐ after each birth
- ☐ more than once a week
- ☐ less than once a week

How long do calves stay with their dams after calving?

- ☐ <1h
- ☐ 1-3h
- ☐ >3h

Co-husbandry of calving and dry cows?

- ☐ yes, in more than half of the calvings
- ☐ yes, in less than half of the calvings
- ☐ no

Co-husbandry of calving and sick cows?

- ☐ yes, in more than half of the calvings
- ☐ yes, in less than half of the calvings
- ☐ no

Calves up to 28d

Housing:

- ☐ single Igloo (____%) ☐ single pen (____%)
- ☐ group Igloo (____%) ☐ group pen (____%)

■Change of the housing:

- ☐ 2 weeks
- ☐ no change
- ☐ _____

New housing:

- ☐ group igloo (____%)
- ☐ group pen (____%)

■Floor:

- ☐ concrete floor
- ☐ natural floor

■ Bedding material and frequency:

- ☐ straw
- ☐ daily bedding
- ☐ bedding if necessary

■ Direct contact to other calves:

- ☐ yes
- ☐ no

■ Number of calves per group:

- ☐ calve-feedlot Ratio >1:1
- ☐ calve-feedlot Ratio <1:1

■ Direct contact to other animals

- ☐ yes
- ☐ no
- ☐ _____ (species)

■ All-in-all-out-principle

- ☐ yes
- ☐ no

If yes,

- ☐ all calves
- ☐ not for all calves (____%)

Feeding:

■ Colostrum feeding:

- ☐ immediately after birth
- ☐ until 3 h after birth
- ☐ later than 3 h

☐ colostrum of the own dam (☐ milked off ☐ directly from the dam)

☐ colostrum stored frozen and defrosted

☐ colostrum stored in the refrigerator

☐ colostrum is pasteurized

☐ colostrum is acidified (with_____)

☐ the dams have been present on the farm for at least 3 weeks before birth

☐ foreign colostrum is occasionally used

origin farm: _____

How often? _____ How many calves? _____)

☐ check of colostrum quality

How? _____

How much colostrum do calves receive? _____

How?

☐ drench

☐ nursing bucket

Further feeding management:

☒ ☐ all calves ☐ heifer calves

Age: from _____ to _____

☐ raw milk

☐ waste milk (antibiotic residues)

☐ waste milk (without antibiotic residues)

☐ milk replacer

☐ ___ x per day, _____ (volume)

☐ ad libitum

☐ milk is pasteurized

☐ milk is acidified

Age: From _____ to _____

☐ raw milk

☐ waste milk (antibiotic residues)

☐ waste milk (without antibiotic residues)

☐ milk replacer

☐ __ x per day, _____(volume)

☐ ad libitum

☐ milk is pasteurized

☐ milk is acidified

■ ☐ male calves

Age: From _____ to _____

☐ raw milk

☐ waste milk (antibiotic residues)

☐ waste milk (without antibiotic residues)

☐ milk replacer

☐ __ x per day, _____(volume)

☐ ad libitum

☐ milk is pasteurized

☐ milk is acidified

Age: From _____ to _____

☐ raw milk

☐ waste milk (antibiotic residues)

☐ waste milk (without antibiotic residues)

☐ milk replacer

☐ ___ x per day, _____(volume)

☐ ad libitum

☐ milk is pasteurized

☐ milk is acidified

■Feeding practice?

☐ always at fixed times

☐ irregularly

☐ use of automatic feeder (☐ freshly prepared, ☐ stored)

■How is the calf feeder cleaned and disinfected?

☐ no cleaning and disinfection

☐ with water only

☐ with cleaning agent

☐ with cleaning and disinfectant

How often is the calf feeder cleaned and disinfected?

☐ after each meal

☐ 1 x / day

☐ < 1 x / day

■Do calves receive supplementary feed?

☐ yes, roughage from day _____

☐ yes, concentrate from day _____

☐ no

What is used to prepare the milk replacer?

☐ tap water

☐ well water

■Do the calves have free water access?

☐ yes ☐ no

- ☐ tap water
- ☐ well water

■ Which people take care of the calves?

- ☐ number _____
- ☐ trained ☐ untrained
- ☐ changing ☐ constant

Use of protective clothing?

- ☐ yes
- ☐ no

■ Use of preventive treatment for calves?

- ☐ yes,

- ☐ no

How do you assess calf health? What are the biggest problems?

What do you do about it?

Use of treatment schedules for calves?

- ☐ yes
- ☐ no

Cows

Housing systems:

- ☐ cubicle housing system (____%)
- ☐ deep-bedded straw yard housing system (____%)
- ☐ flat floors (____%) ☐ slatted floors (____%)
- ☐ high cubicle beds (____%) ☐ low cubicle beds (____%)
- ☐ straw (____%)
- ☐ sawdust (____%)
- ☐ _____
- ☐ lime (____%)
- ☐ pasture (____%)

Dry-off-management:

Cows are dried with antibiotic treatment _____(name)

Use of teat sealant?

- ☐ yes
- ☐ no

Cows are dried _____ days/weeks before birth date.

Use of maternal vaccinations?

- ☐ yes, Rota/Corona/E.coli
- ☐ yes, _____
- ☐ no

Milking routine:

Udder preparation:

- ☐ dry cleaning
- ☐ wet cleaning _____
- ☐ pre-dip
- ☐ milked by hand
- ☐ stimulated per brush _____

Use of post-milking treatment

☐ yes, with _____

☐ dip

☐ spray

Use of milking cluster disinfection

☐ yes, with _____

☐ no

Use of separate milking parlor for sick cows?

☐ yes

☐ no

How is the milking order?

☐ cows with waste milk at the end

☐ cows with high somatic cell count at the end

☐ mixed order

Health management:

Use of preventive treatment for cows?

☐ yes, _____ (____%)

☐ no

Use of treatment schedules for cows?

☐ yes

☐ no

Is every clinical mastitis treated with antibiotics?

☐ yes, with _____ (____%)

☐ no

Other treatment schedules:

If a treatment doesn't improve the health of the animal, when do you change the antibiotic treatment?

- ☐ never (____%)
- ☐ after 1 day (____%)
- ☐ after 2 days (____%)
- ☐ after 3 days (____%)
- ☐ only after completion of the treatment (____%)

Use of alternative treatment methods, e. g. homeopathy?

- ☐ yes, _____ (____%)
- ☐ no

Housing of the cows producing waste milk?

- ☐ in the regular group (____%)
- ☐ separated with other waste milk cows (____%)
- ☐ separated with other cows, that were treated with antibiotics (____%)

Cleaning and disinfection management:

How does the cleaning and disinfection proceed?

Calving pen	Calf housing	Milking parlor
<p>Cleaner</p> <p><input type="radio"/> we do not use any cleaning agent</p> <p>Exposure time</p> <p><input type="radio"/> shorter than the manufacturer's instructions</p> <p><input type="radio"/> according to the manufacturer's instructions</p>	<p>Cleaner</p> <p><input type="radio"/> we do not use any cleaning agent</p> <p>Exposure time</p> <p><input type="radio"/> shorter than the manufacturer's instructions</p> <p><input type="radio"/> according to the manufacturer's instructions</p>	<p>Cleaner</p> <p><input type="radio"/> we do not use any cleaning agent</p> <p>Exposure time</p> <p><input type="radio"/> shorter than the manufacturer's instructions</p> <p><input type="radio"/> according to the manufacturer's instructions</p>
Is there complete dry-through?	Is there complete dry-through?	Is there complete dry-through?

<input type="radio"/> yes <input type="radio"/> no	<input type="radio"/> yes <input type="radio"/> no	<input type="radio"/> yes <input type="radio"/> no
Disinfectant <input type="radio"/> we do not use a disinfectant Exposure time <input type="radio"/> shorter than the manufacturer's instructions <input type="radio"/> according to the manufacturer's instructions	Disinfectant <input type="radio"/> we do not use a disinfectant Exposure time <input type="radio"/> shorter than the manufacturer's instructions <input type="radio"/> according to the manufacturer's instructions	Disinfectant <input type="radio"/> we do not use a disinfectant Exposure time <input type="radio"/> shorter than the manufacturer's instructions <input type="radio"/> according to the manufacturer's instructions
Use of cleaning and disinfection plan <input type="radio"/> yes <input type="radio"/> no	Use of cleaning and disinfection plan <input type="radio"/> yes <input type="radio"/> no	Use of cleaning and disinfection plan <input type="radio"/> yes <input type="radio"/> no
Cleaning agents? <input type="radio"/> Surfactants <input type="radio"/> Acids <input type="radio"/> Bases <input type="radio"/> Bleaching agents <input type="radio"/> Enzymes	Cleaning agents? <input type="radio"/> Surfactants <input type="radio"/> Acids <input type="radio"/> Bases <input type="radio"/> Bleaching agents <input type="radio"/> Enzymes	Cleaning agents? <input type="radio"/> Surfactants <input type="radio"/> Acids <input type="radio"/> Bases <input type="radio"/> Bleaching agents <input type="radio"/> Enzymes
Disinfectants? <input type="radio"/> Peracetic acid <input type="radio"/> Hydrogen peroxide <input type="radio"/> Chlorine <input type="radio"/> Iodine <input type="radio"/> Aldehydes <input type="radio"/> Alcohols	Disinfectants? <input type="radio"/> Peracetic acid <input type="radio"/> Hydrogen peroxide <input type="radio"/> Chlorine <input type="radio"/> Iodine <input type="radio"/> Aldehydes <input type="radio"/> Alcohols	Disinfectants? <input type="radio"/> Peracetic acid <input type="radio"/> Hydrogen peroxide <input type="radio"/> Chlorine <input type="radio"/> Iodine <input type="radio"/> Aldehydes <input type="radio"/> Alcohols

<input type="radio"/> Phenols <input type="radio"/> Nitrogen compounds	<input type="radio"/> Phenols <input type="radio"/> Nitrogen compounds	<input type="radio"/> Phenols <input type="radio"/> Nitrogen compounds
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Assessment of the herd manager

What is the biggest health problem of the herd?

How many animals do you treat with antibiotics per day on average?

- ☐ <2 per 100 animals treated
- ☐ 2-5 per 100 animals treated
- ☐ 5-10 per 100 animals treated
- ☐ >10 per 100 animals treated

Which antimicrobial ingredients were used in the farm in the last 6 months?

- ☐ β -lactam antibiotics (☐ penicillins ☐ cephalosporins)
- ☐ tetracyclines
- ☐ aminoglycosides
- ☐ Polypeptides
- ☐ Macrolides
- ☐ Fenicol
- ☐ Folic acid antagonists
- ☐ Chinolons