

**Supplementary file S1.** Questionnaire of the study multi-drug resistant extended-spectrum betalactamase (ESBL)-producing Escherichia coli in a dairy herd: distribution and antimicrobial resistance profiles.

Data of the participants

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**I: General questions: breeding organization**

**Who takes care of the cattle?**

- The owner of the herd
- The family member(s)
- The employee(s)
- The contractor(s)

**How many.... are present on the farm?**

Breeding bulls	Dairy cows	Dry cows	Heifers	Weaned calves	Suckling calves
0	457	86	254	153	50

**How many milking groups are there, and how are they divided (fresh/fresh/tight)?**

4 groups (primiparous - early - mid-late stage cows)

**Do you buy animals?**      No

**Upon entering the farm, do you have to put...?**

	Always	Sometimes	Never
Specific clothing	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>
Single use clothing	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>

**II: Health management in the sick pen and management outbreaks**

**Are sick animals physically isolated from the rest of the herd?**

- Always
- Sometimes
- Never

**Are there specific equipment and materials (e.g., buckets, thermometers, cleaning and feeding utensils, gastric probes...) in the area used for sick animals?**

- Yes
- No

**Is this specific equipment cleaned and disinfected before a new animal enters the sick animal area?**

- It is cleaned and disinfected
- It is only cleaned
- No

**Are cattle in the area used for sick animals wholly separated from the rest of the herd?**

- Yes
- No

**I... before entering the area used for sick cattle**

	Always	Sometimes	Never
Use specific boots	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Wear specific clothing	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Wash hands/use gloves	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>

**Is the area used for sick animals empty after each use?**

- Yes
- No

**The area used for sick animals is...before each new introduction of other sick animals**

	Always	Sometimes	Never
Cleaned	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Disinfected	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dried	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**Do you care for sick livestock before or after healthy livestock?**

- Before
- After
- No specific order

**Can a group of sick cattle be separated from the rest of the herd in case of an epidemic?**

- Always
- Sometimes
- Never

**Is a record kept of animal health data?**

- Yes
- No

**Are there written protocols for vaccination, disease treatment, and hygiene procedures?**

- Yes
- No

**Is a bacteriological examination of the udder of all cows performed regularly (i.e., at least once a year)?**

- Yes
- No

**What happens to animals identified as carriers of disease?**

- Elimination (always)       Elimination (sometimes).
- Segregation (always)       Segregation (sometimes)
- Nothing (always)       Nothing (sometimes)
- Delayed elimination (always)       Delayed elimination (sometimes)

**Litter type used in the sick pen:** Straw

### **III: Reproduction management**

**Are calving boxes available on the farm?**

- Yes, there are individual calving boxes
- Yes, there are shared calving boxes
- No

**Has the calving box ever been used to house sick animals, or is it adjacent to areas used for that?**

- Always
- Sometimes
- Never

**Are the cows in the calving box completely separated from the other animals?**

- Yes
- No

**Are... in the calving box?**

	Always	Sometimes	Never
<b>Compartment-specific boots worn</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
<b>Compartment-specific clothing worn</b>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Hands washed/(new) gloves used</b>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**The calving box is ... before the introduction of new animals?**

	Always	Sometimes	Never
Cleaned	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Disinfected	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Dried	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

**When helping with deliveries/abortions, are the hands and obstetric materials used always cleaned and disinfected before and after each delivery/abortion?**

- They are cleaned and disinfected
- They are only cleaned
- No

**When does the separation of the calf from the mother take place?**

- Within one hour of the calf's birth
- No separation; the calf remains with the mother as a suckling calf
- Just after birth

**If an abortion occurs, is the cow tested (i.e., abortion protocol)?**

- Always
- Sometimes
- Never

**Where are fetal tissues and membranes disposed of after a birth/abortion?**

- Are they left in the barn/delivery area or where the abortion occurred
- They are eaten by the cow/dog/other
- They are put on the manure pile/slurry pit
- Other

**Type of bedding used and how often changed:** Straw, changed when necessary (evaluation of dirtiness)

#### **IV: Calf rearing**

**How many colostrum meals do they make?** 3: The first with the mother, one or two the day after

**How many milk meals do they take?** Two each day

**How many liters of colostrum are given to the calf in the first six hours after birth?** Ad libitum

**How is the colostrum administered?**

- Directly from the mother
- Bottle
- Bucket
- Probe

**Does the mother give colostrum, or is the colostrum bank used on the farm?**

There is no colostrum bank, and it is given by the mother

**Is it checked whether the quality of the colostrum is sufficient?**

Yes and method: refractometer

No

**Is there a frozen or artificial supply of colostrum in case the mother does not provide enough milk, or the colostrum has insufficient quality?**

No

Yes, there is a frozen supply from other cows on my farm

Yes, there is a frozen reserve from outside my farm

Yes, there is an artificial reserve

**If colostrum is not fed to the calf immediately after milking, is the colostrum stored in a refrigerator?**

Yes

No

**Are the materials used for colostrum administration (e.g., test tubes, bottles, etc.) cleaned and disinfected after each use?**

They are cleaned and disinfected

They are only cleaned

No

**Are calves fed waste milk (i.e., milk not suitable for the bulk tank milk)?**

Always (only the male calves)

Sometimes (specify cases)

Never

**If they give waste milk, what type is it?**

High cells

Antibiotic

Colostrum/transitional milk

**Is the waste milk pasteurized? If yes, according to what process?**

Yes, at 65 °C for 30 minutes (after that, it remains for 12 hours and heat when needed at 40°C)

**If pasteurizer is used, does it undergo washing processes?**

Yes, only with water – no disinfectant

**To whom is the waste milk fed, and for how long?**

To the male calves until they are sold

**List the division of calf groups from birth to weaning**

Males and females in single pens; females in boxes with the calf feeder machine

**V: Calf housing**

**Are calves housed in individual pens/boxes or separate areas?**

Yes

No

**Are individual pens/calf boxes/separate areas empty after each use?**

Yes

No

**Are the individual pens/calf stalls/separated areas ... before each new introduction of calves?**

	Always	Sometimes	Never
Cleaned	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Disinfected	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Dried	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**Is calf contact between different group pens/single stalls/separate areas possible?**

Yes

No

**How large (number of animals) are the groups of calves that are grouped from individual pens/separated areas to group pens? 20>40**

**Are the group pens empty after each use?**

Yes

No

**Is the group enclosure (including single pens) ... before each new introduction of calves?**

	Always	Sometimes	Never
Cleaned	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
Disinfected	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
Dried	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>

**Are the single pens disinfected?**

Single pens are disinfected only in the spring/summer/autumn period but not in winter

**During the same feeding session, are milk-feeding buckets/teats reused between calves?**

Yes

No

**Are the feeding buckets cleaned after each feeding session?**

Yes

No

**Is there a feeding order for calves?**

Male calves are fed first, then females from the same buckets without washing them.

## **VI: Health management of the herd**

**Do you use selective dry cow therapy?** No, all the cows receive blanket dry cow therapy with cefalonium dihydrate

**Mastitis predominantly pathogens:** Environmental pathogens

**What antibiotics do you use for mastitis?** Amoxicillin/clavulanic acid

**Which antibiotics do you use to treat diarrheic calves?** Gentamicin in the case of *E. coli*

**Microbiological fecal control of calves with diarrhea?**

Yes (how many times/year \_\_\_\_\_)

No

**Do you perform *Cryptosporidium* spp. prevention?** No

**Do you vaccinate dry cows for neonatal calf diarrhea?**

Yes: Rota-Corona

No

**Do you vaccinate calf for bronchopneumonia?**

Yes

No

**Do you vaccinate for BVD?**

Yes

No

**IBR herd status:**

IBR free

IBR vaccination

Unknown

**Is the drinking water quality checked annually at the source or storage tank by bacteriological analysis?**

Yes

No, but I am using a water supply

No

**Is the drinking water quality checked annually at the main distribution points (watering source for livestock) by bacteriological analysis?**

Yes

No

**How often do you change the water in the calf buckets?**

Always

Sometimes (daily or every two days)

Never

**Are the cow water troughs cleaned?**

Always

Sometimes

Never

## **VII: Milking management**

**Do you use a milking parlor or a milking robot?**

- Milking robot
- Milking parlor

**How many times/year is performed a static measurement of the milking equipment? 2**

**How many cows are milked on average? 380**

**How many milkings are performed per day? 2**

**How many milking positions are in the milking parlor? 24 + 24**

**Do you use rubber or silicon liner liners?**

- Rubber liners
- Silicon liners

**Are milking clusters disinfected between cows?**

- Yes, after each cow
- Yes, after cows with mastitis
- Yes, after cows with high somatic cell count (SCC)
- No

**How are milking clusters disinfected between animals?**

- With hot water (more than 75°C)
- With steam
- Other

**Are teats cleaned before milking? If yes, how?**

- Yes, pre-dipping
- Yes, dry cleaning with separate towels
- Yes, wet washing and drying afterward with separate towels
- Yes, wet washing, but not drying afterward (disposable disinfectant wipes)
- No

**Is the first milk examined during preliminary extraction?**

- yes
- no

**Are the teats disinfected after being milked?**

- Yes, with dipping
- Yes, with a spray
- No

**Are cows kept at the station for a while after milking?**

- Yes, from 30 minutes to one hour
- Yes, for more than one hour
- No

**Are the cows milked in a specific order?**

- Yes, cows with mastitis and/or high SCC are milked last
- Yes, cows with mastitis and/or high SCC are milked first
- No

**Are the udders of lactating cows shaved?**

- Yes, two or more per year
- Yes, once a year
- No

**Are the tails of lactating cows shaved?**

- Yes, two or more times a year
- Yes, once a year
- No

**What type of bedding do you use for lactating cows?** Straw in the cattle cubicle