TAFS

TRUST IN ANIMALS AND FOOD SAFETY

a non-profit Swiss Foundation

TAFS White Paper

Antimicrobial residues in condemned (waste) milk and its potential impact on public health/food safety

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1. Executive summary

Every application of AM in human and veterinary medicine deserves scrutiny with the aim of insuring the most judicious use in order to limit the rate of emergence of AMR pathogens. This review focuses on a specific application of AM, namely the use of AM for mastitis control in dairy cattle and the consequences of exposure of calves fed AM-contaminated milk. Most AM used in dairy herds is for mastitis treatment and management and most of the AMs used qualify as medically important antimicrobial drugs. As such, their use in animals should be very carefully considered and justified. Use of AM for mastitis treatment and prevention is necessary for assuring animal health. It is crucial, however, that these AM continue to be used judiciously by having direct involvement of veterinarians.

Milk from cows given AM for mastitis treatment and prevention cannot enter the food supply. This so called waste milk is then often fed to calves. Feeding raw (unpasteurized) waste milk to calves is discouraged by veterinarians due to infectious disease transmission risks. However, pasteurization is unlikely to affect the activity of most AMs. The levels of AM in this waste milk are very low but can contribute to emergence of resistant bacteria. However, this may not represent a significant contribution to AMR at the, regional or national-level when compared with other AM uses in animal agriculture. There are other practices, such as the use of mediated milk replacers for calf feeding, and extensive AM use in the veal calf industry that are well known to contribute to AMR in pathogens found in the intestines of calves which deserve more attention.

2. *Introduction*

The previous TAFS white paper covers the broader issues of antimicrobial (AM) usage and antimicrobial resistance (AMR) in food-producing animals (http://www.tafsforum.org/other-topics.html). The scope of this second white paper is restricted to the impact of antimicrobials occurring in milk from dairy cattle being treated for intramammary infections (IMI), also simply known as mastitis, which is not suitable for human consumption and is therefore fed to calves.

3. *Mastitis background*

Mastitis is an occupational hazard for dairy cattle. Dairy cattle have been selectively bred for centuries to produce large quantities of milk. A typical dairy cow produces 24-28 liters of milk each day. The bovine udder is thus large and holds a rich substrate able to support the growth of many microbes. The cow's teats, more specifically the end of the teat canal, is the only barrier between the myriad microbes in the cow's environment and the milk-filled mammary tissue. The act of milking, which happens two or three times daily, relaxes the teat sphincter (muscle surrounding the teat end) opening the teat canal allowing the flow of milk. This also compromises this critical microbial barrier making it easier for microbes to invade.

There are many routine practices on farms designed to minimize mastitis. Cows are housed in a manner to keep them as clean as possible. Particular attention is paid to their bedding as this comes in direct contact with the cow's teats. In preparation for milking the teats of cows are disinfected prior to application of the milking equipment. The equipment is flushed with water after each cow is milked to rinse away milk and remove microbial contaminants. Finally, the teats are again disinfected after milking has finished and the equipment has been removed. The cow's udder remains vulnerable to infection for a period of time after milking while the teat end dries, the teat sphincter contracts, and a plug of keratin forms to block the teat canal. This is nature's way of protecting the udder from infection. Ideally, cows are managed in a way that keeps them standing during this post-milking period so as to minimize exposure to environmental bacteria; particularly those in the cow's bedding.

Mastitis is the most common infectious disease of dairy cattle. It has a range of causes but most are bacterial with the most common being *Staphylococcus* species, *Streptococcus*

species, and *Escherichia coli*. Intramammary infections can begin at any time during the lactation cycle; that is, during lactation, which begins after a cow gives birth to a calf and lasts roughly 305 days, or during the non-lactating part of the cycle, referred to as the dry period, lasting roughly 60 days. The cow's immune system responds to bacterial infections by sending white blood cells to the udder to kill the invading microbes. Such cells originating from the cows, not the infecting microbes, are called somatic cells. Counting somatic cells in milk, called somatic cell counts (SCC), is the most common measures of udder health. High counts indicate probable mastitis.

Clinical mastitis is defined as an IMI that causes a visible alteration in the milk (clotted, bloody or stringy), to the udder (edema, redness, swelling), or when the cow has a fever or other systemic signs of illness, in addition to a rise in somatic cell counts (SCC) in the milk. Subclinical mastitis is only evident by an increase in SCC. Values above 200,000 cells/mL of milk in composite milk samples of 4 quarters are generally considered indicative of mastitis, although there are regional and herd-level reasons why this is not considered an absolute cutoff for defining a case of mastitis.

Somatic cell counts are also directly related to the quality and quantity of dairy products made from milk. For this reason, dairy processors desire milk from herds with a low SCC and commonly pay dairy producers a premium for milk from low SCC herds based on testing the pooled milk in the dairy herd's storage tank, also called bulk tank. This provides a strong financial incentive for dairy producers to continuously strive to limit mastitis in their dairy cattle.

4. *Antimicrobials for mastitis control*

Antimicrobials are used for both the treatment and prevention of mastitis. Treatment of cows ending their lactation cycle, called dry-cow therapy, is the most frequent usage of AM for mastitis control in dairy herds. These treatments are administered through the teat as intramammary infusions to attain the highest possible AM concentration in the cow's udder without exposing the entire animal to

antibiotics. Although there are variations in this practice among countries, it is common to administer dry-cow therapy to 100% of cows in dairy herds due to the high risk of infection during this period. Because this treatment both cures existing IMI and prevents new IMI during the cow's dry (non-lactating) period, this practice is deemed beneficial for animal health, welfare and longevity, as well as farm productivity and milk quality. However, it should be noted that effective dry-cow is important in the larger scheme because: 1) it lowers the incidence of mastitis during lactation thereby lessening the amount of AM needed for treatment of mastitis in lactating cows and therefore the amount of AM that might be in milk fed to calves on the farm, and 2) after dry cow therapy there is little or no AM remaining in colostrum or milk when the cow starts lactating.

Most of the AM used for mastitis control are drugs that are "medically important antimicrobials"; meaning that they are also used for treating infections in humans. Medically important antimicrobials used in veterinary medicine are under increased scrutiny as their use in animals could potentially lead to more AMR in pathogens affecting humans. The OIE publication outlines a broad international approach to dealing with the issue (The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials, November 2016). The U.S. FDA has two very specific principles regarding judicious use of AM in animals (FDA Guidance for Industry #209, April 13, 2012).

Principle 1: The use of medically important antimicrobial drugs in food-producing animals should be limited to those uses that are considered necessary for assuring animal health.

Some may have concerns that the use of medically important antimicrobial drugs in food-producing animals for disease prevention purposes is not an appropriate or judicious use. However, FDA believes that some indications for prevention use are necessary and judicious as long as such use includes professional veterinary involvement. Veterinary involvement in the decision-making process associated with the use of medically important antimicrobial drugs is an important aspect of assuring appropriate use, including judicious prevention use.

Principle 2: The use of medically important antimicrobial drugs in food-producing animals should be limited to those uses that include veterinary oversight or consultation.

These principles broadly apply to use of antimicrobials in veterinary medicine in all countries of the world.

Typically, mastitis, whether clinical or subclinical, is diagnosed by the dairy producer with the help of milk SCC information and the appearance of the milk. Seldom are microbiological cultures done to determine the exact cause. Figure 1 (from Reugg, 2015) illustrates the diversity of microbiological results for 793 cases of clinical mastitis in 20 Wisconsin USA dairy herds. European studies report comparable results with Staphylococcus aureus, Streptococcus uberis and E. coli being the predominant pathogens isolated (Thomas, 2015). AM that target gram-positive bacteria such as penicillin or pirlimycin, are warranted when mastitis is caused by Staphylococcus aureus, coagulase-negative Staphylococcus species (CNS), or Streptococcus species (labeled Env. Strep. on the graphic). Collectively these caused 19% of clinical mastitis cases in the 20 study herds. AM that target gram-negative bacteria are warranted when mastitis us due to E. coli, Klebsiella, or Enterobacter (31% of cases shown on the graphic). If a milk sample yields "no growth" by microbiological analysis (26% of cases in this study) use of AM for treatment is not justified.

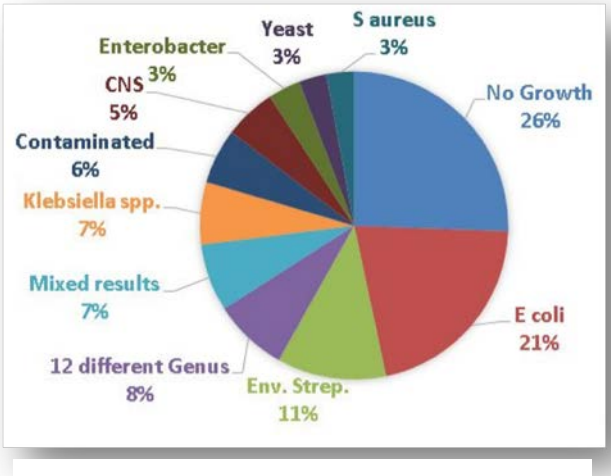

Figure 1

Rather than investigate the cause of mastitis, a broad-spectrum antimicrobial, one that acts on both gram-positive and gram-negative bacteria such as ceftiofur, is sometimes is selected (Reugg, 2015). In the study of 20 Wisconsin dairy farms selected based on a bulk tank SCC >250,000 cells/mL, Reugg (2015) found that roughly 80% of all AM used on dairy farms were used for treatment or

prevention of mastitis: 28% for intramammary dry-cow therapy, 38% for intramammary treatment of clinical mastitis, and 17% as injectable AM for clinical mastitis. The estimated overall exposure to AM was 5.43 defined daily doses per cow per year.

One would assume that use of AM on farms will, over time, lead to more AMR. However, this is very dependent on the specific AM in question and the mechanisms by which bacteria become resistant. Pol & Reugg (2007) reported that resistance of mastitis pathogens to pirlimycin was directly related to the use of this AM on farms, as quantified by defined daily doses. Exposure to most other AM, notably the commonly used drug cephapirin, was not associated with emergence of resistance as defined by the minimal inhibitory concentrations (Pol & Reugg, 2007). A Canadian study reported an association between herd-level AM use and AMR in bovine mastitis coliforms for certain antimicrobials. Differences in AMR between different barn types and geographical regions were not observed (Saini, 2013).

5. *Antimicrobial residues in cow's milk*

Cows treated with AM for mastitis or any other reason will have AM residues in their milk and meat. The duration of time when residues can be detected is dictated by the pharmacokinetics of the specific antibiotic in question; the rates and routes of drug excretion. Pharmacokinetic data are provided by AM manufacturers as part of the drug registration process. To avoid AM residues in food, governmental regulations define the time after which a cow is treated with AM when the milk and meat must not be allowed into the food chain. These times are called withholding times. In addition to the chemical nature of the AM, withholding times differ depending on the route of AM administration, e.g. oral, intramammary, or injection, and whether the cow is lactating or not, i.e. a "dry cow". Tables 1-3 list the US FDA withholding times for AM drugs used in dairy cattle. These same drugs

are also legal for use in the EU but a few additional drugs may be legal in some countries that are not found on Table 1-3. A comparison of antimicrobials legal for use in dairy cattle by country was not found. A more general list of AMs licensed for use in the EU can be found in Table 3 of the recent FAO publication (Wall et al, 2016). The tables in this paper are primarily for illustration purposes.

Table 1. Common Antimicrobial Drugs for Intramammary Use in Non-lactating cattle. Adapted from "Milk and Dairy Beef Drug Residue Prevention". National Milk Producers Federation. 2012

Active ingredient	Drug Type	Withholding Time		Product	
		Milk	Meat	Brand Name	Manufacturer/Marketer
Ceftiofur hydrochloride	Rx	None	30 days	Spectramast™ DC	Pfizer
Cephapirin	OTC	72 hours	42 days	Tomorrow Infusion	Boehringer Ingelheim
Cloxacillin	Rx	None	30 days	Dry-Clox®	Boehringer Ingelheim
	Rx	None	28 days	Orbenin-DC®	Merck Animal Health
Novobiocin	OTC	72 hours post-calving	30 days	BioDry®	Pfizer, Inc.
Penicillin G (procaine)	OTC	72 hours post-calving	14 days	Hanford's/ US Vet go-dry™	G.C. Hanford Mfg. Co.
Penicillin G + Dihydrostreptomycin	Rx	96 hours post-calving	60 days	Quartermaster® Dry Cow Treatment	Pfizer Inc.
Penicillin G + Novobiocin	OTC	72 hours post-calving	30 days	AlbaDry® Plus Suspension	Pfizer, Inc.

Rx: Prescription

OTC: Over-the-counter (direct sales to dairy producers)

Note: Chlortetracycline, monensin, and neomycin sulfate are antimicrobial drugs approved for oral administration in non-lactating cattle. While they have meat withholding times, they do not have milk withholding times and so presumably have no significant milk residues of concern when feeding milk from treated cows to calves.

Table 2. Common Antimicrobial Drugs for Intramammary Use in Lactating cattle. Adapted from "Milk and Dairy Beef Drug Residue Prevention". National Milk Producers Federation. 2012

Active ingredient	Drug Type	Withholding Time		Product	
		Milk	Meat	Brand Name	Manufacturer/Marketer
Amoxicillin	Rx	60 hours	12 days	Amoxi-Mast®	Merck Animal Health
Ceftiofur hydrochloride	Rx	72 hours	2 days	Spectramast™ LC	Pfizer, Inc.
Cephapirin	OTC	96 hours	4 days	Today®	Boehringer Ingelheim
Cloxacillin	Rx	48 hours	10 days	Dairyclox®	Merck Animal Health
Hetacillin	Rx	72 hours	10 days	Hetacin®K	Boehringer Ingelheim
Penicillin G (procaine)	OTC	60 hours	3 days	Hanford's/ US Vet MASTICLEAR™	G.C. Hanford Mfg. Co.
Pirlimycin	Rx	36 hours	9 days	Pirsue®	Pfizer Inc.

Rx: Prescription

OTC: Over-the-counter (direct sales to dairy producers)

Note: Sulfamethoxine (Naquasone Bolus; Merck Animal Health) is approved in the U.S. for oral administration to lactating cows if prescribed by a veterinarian. It has a 72 hour milk withholding time and no meat withholding time.

Table 3. Common Antimicrobial Drugs for Injectable Use In Lactating cattle. Adapted from "Milk and Dairy Beef Drug Residue Prevention". National Milk Producers Federation. 2012

Active ingredient	Drug Type	Withholding Time		Product	
		Milk	Meat	Brand Name	Manufacturer/Marketer
Ampicillin	Rx	48 hours	6 days	Polyflex®	Boehringer Ingelheim
Ceftiofur crystalline	Rx	None	13 days	EXCEDE®	Pfizer, Inc.
Ceftiofur hydrochloride	Rx	None	3 days	EXCENEL® RTU	Pfizer, Inc.
Ceftiofur sodium	Rx	None	4 days	Naxcel® sterile powder	Pfizer, Inc.
Oxytetracycline	OTC	96 hours	28 days	Agrimycin 200	Agri Laboratories, Inc.
	OTC	96 hours	28 days	Bio-Mycin® 200	Boehringer Ingelheim
	OTC	96 hours	28 days	Oxytetracycline injection 200	Norbrook Laboratories Ltd
	OTC	96 hours	28 days	Pennox 200 injectable	Pennfield Animal Health
	OTC	96 hours	28 days	Liquamycin® LA-200®	Pfizer, Inc.
Penicillin G (procaine)	OTC	48 hours	10 days	Agri-Cillin injection	Agri Laboratories, Inc.
	OTC	48 hours	4 days	Pro-Pen-G™ injection	Bimedia, Inc.
	OTC	48 hours	10 days	Sterile Penicillin G	Norbrook Laboratories Ltd
	OTC	48 hours	14 days	Narcillin	Norbrook Laboratories Ltd
Sulfadimethoxine	Rx	24 hours	5 days	Di-Methox injection 40%	Fort Dodge Animal Health

Rx: Prescription

OTC: Over-the-counter (direct sales to dairy producers)

6. *Antimicrobial residue levels in milk fed to calves*

During the milk withholding period, low levels of AM will be found in milk. To avoid human exposure to these residues, the AM-treated cow's milk is not comingled with that of the rest of the dairy herd. This milk thus considered not "saleable", it is often called "waste milk". Rather than simply dump this milk down the drain, waste milk is commonly fed to young calves being raised on the farm either for meat or as replacements for the dairy herd. A survey of dairy farms in England and Wales showed that 83% of 413 survey respondents fed waste milk to calves. Of these, 87% fed waste milk from cows with mastitis, and only one-third discarded the first milk after antibiotic treatment (the milk likely to contain the highest level of AM). In the U.S. 33.4% of farms fed waste milk to dairy calves and all but a small percentage did so without first pasteurizing the waste milk (NAHMS, 2012). Little is known about the effect of pasteurization on AMs but while this process effectively kills most microbial pathogens, it probably has little effect on the activity of the AMs.

Some calves may be fed the milk collected soon after a lactating cow was treated for mastitis with an AM administered by the intramammary route and so the milk from the treated quarter may have a moderately high level of AM. Commonly, only one or two quarters might develop mastitis while the others remain healthy. Only the quarters with mastitis are treated but all of the milk from the cow will be considered waste milk. Calves are fed milk pooled from all four quarters of multiple cows, only some which have been AM-treated for varying times prior to milk collection, e.g. 0 to 96 hours based on the AM milk withholding times. Thus, it is problematic to estimate with any degree of certainty what level of AM calves fed waste milk are typically consuming. It seems reasonable to assume that it is quite low but probably detectable by existing drug residue assays.

The CODEX Alimentarius website lists the maximum residue limits (MRL) for many AM in food products:http://www.fao.org/fao-who-codexalimentarius/standards/vetdrugs/veterinary-drugs/en/. Others can be found at the European Medicines Agency website: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/vet_mrl_search.jsp&mid=WC0b01ac058006488e

The level of AM in any milk fed to calves is likely at or slightly above the MRL (Table 4).

Table 4. Maximum residue limits in milk for intramammary antimicrobials used in dairy.

Antimicrobial	Maximum residue limits
Ceftiofur	100 µg/Kg
Cephapirin	10 µg/Kg
Cloxacillin	30 µg/Kg
Novobiocin	50 µg/Kg
Penicillin	4 µg/Kg
Pirlimycin	200 µg/Kg

7. *Consequences of feeding AM to calves*

The bulk of the scientific literature on the consequences of feeding AM to calves is directed at the practice of using milk replacer products containing oxytetracycline and neomycin, generally referred to as medicated milk replacers. In the U.S. 57.5% of dairy herds used medicated milk replacer to feed calves (NAHMS, 2007). This practice selects for bacteria with resistance to the oxytetracycline and neomycin in the medicated milk replacer as well as other antimicrobials, such as the aminoglycosides, chloramphenicol, and sulfonamides (Berge et al. 2006). Experimentally, emergence of AMR to penicillin was directly related to the level of penicillin fed in milk to dairy calves (Langford et al., 2003). On a more encouraging note, Kaneene et al. (2008) demonstrated that the impact of medicated milk replacer on AMR is reversible: termination of use of medicated milk replacer on farms resulted in a return to AM susceptibility for E. coli and Salmonella isolated from dairy calves. Berge et al. (2006) showed that there was little or no environmental transfer of resistant traits or bacteria between calves.

However, a very recent Swedish demonstrated probable the within-farm and among farm spread of quinolone-resistant E. coli (Duse, 2016)

It is logical to assume that AM in waste milk can foster emergence of AMR in the gut of calves, both to the specific AM in the waste milk and possibly to other AM. A Swedish study (Duse et al., 2015) nicely demonstrated this for one organism, E. coli isolated from calf feces, for two AMs: streptomycin and nalidixic acid. Noteworthy is that the prevalence of AM resistance to the 10 other AMs tested in that study was not significantly higher for fecal E. coli from calves on farms feeding waste milk. Similarly, a study involving 114 calves demonstrated that feeding waste milk, whether pasteurized or not, was associated with a higher prevalence of resistance of E. coli from calf feces to 3 of the 25 AMs studied when compared with calves fed bulk tank milk (Aust et al, 2013). Notably, this was not true for Enterococcus species bacteria when tested against the same 25 AMs in that study highlighting that study findings such as these should not be generalized across all bacterial types or all antimicrobials. The study by Pereira et al. (Pereira, 2015) further supports that waste milk feeding can influence the frequency of AMR in calves.

Concentrations of AM in waste milk fed to any calf are affected by many farm management factors, e.g. size of herd, rate of mastitis, the use of AMs and choice of AMs to treat mastitis, the time after AM treatment when the waste milk is collected, and whether waste milk is pooled from all sick cows in the herd on a given day or is fed from a single cow to a single calf. In general, selective pressure on some bacteria by certain AM in waste milk can occur but is apparently limited and transitory. Also, with the exception of the veal calf industry, most of these calves will not enter the food chain for several months by which time any AMR induced by the AM-containing waste milk will be undetectable and of no consequence to consumers. Lastly, if the waste milk is discarded, the selective pressure on microbes to become resistant will simply occur in the environment (soil, sewer, or septic system) into which the waste milk is discarded rather than in the calf gut.

8. *Challenges, opportunities, and areas needing improvement*

1. Educate the scientific community about the importance of dry-cow AM therapy to both treat and prevent mastitis.
2. Improve the level of detail collected about AM usage in dairy herds for national monitoring programs, e.g. dry-cow therapy or lactating cow mastitis or other uses.
3. Continue stressing to dairy producers the importance of mastitis prevention by hygienic milking practices and animal housing systems.
4. Upgrade knowledge of veterinarians about AM and AMR through post-graduate training courses and professional meetings.
5. Restrict or eliminate OTC use of AM to insure that veterinarians are fully engaged in the decisions about when and which AM to use for mastitis treatment and prevention in dairy cattle.
6. Improve on-site microbiological diagnostics to insure that the AM is targeted to the relevant mastitis pathogen; gram-positive or gram-negative bacteria.
7. Design cost-effective herd surveillance tools to monitor which mastitis pathogens predominate on each farm and any seasonal variation in pathogen prevalence.
8. Design herd surveillance tools to objectively determine whether AMR is emerging on a specific farm and if or when the first-choice AM for mastitis treatment should be changed.
9. Foster development of veterinary-specific antimicrobials.

9. *Speculation about the future:*

The term “resistome” has been proposed for the collection of all the antibiotic resistance genes and their precursors in both pathogenic and non-pathogenic bacteria (Wright, 2007). This resistome captures the net effects of AM use on bacteria in any ecosystem, be it the gut of a calf or the floor of a barn. In the future it may be critical to monitor the resistome as a measure of whether antimicrobial

drugs are being used judiciously and the extent to which any farm is contributing to the global problem of AMR.

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