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SYGNET Anti-Bacterial Teat Wipes Field Efficacy Testing

DairyNZ Report

Cox Industries 001

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For: COX Industries Ltd
By: John Williamson
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Background

Cox Industries Ltd, based in Christchurch, is the largest manufacturer of Wet Wipes in NZ providing a range of wipes for both the commercial and domestic markets. Their wipes are marketed under the SYGNET brand along with a number of private labels.
Managing Director David Cox met with John Williamson on 22nd February 2011 to discuss the possibility of Dairy NZ carrying out field trials on the SYGNET brand of Anti-Bacterial Teat Wipes, to determine their efficacy in disinfecting cows' teats prior to the use of dry cow intramammary treatments.

Disinfection of the external teat skin is essential before intramammary products are introduced through the teat canal. The basic aseptic technique requires that, prior to intramammary infusions, teat ends must be clean and dry with a minimal surface population of bacteria. Without proper preparation, organisms present on the teat end can be pushed into the udder and result in intramammary infection and mastitis. This is particularly true when Gram-negative bacteria are introduced into the udder, causing acute mastitis. (NMC Factsheet 2006)

Objective

The proposed work compared the efficacy of two disinfecting procedures in reducing the surface load of bacteria on cows' teats. Experiments were carried out using "natural exposure" conditions on cows from within the Lye Farm Research Herd. Natural exposure refers to the resident population of bacteria present on cows' teats.

Interested parties

COX Industries Ltd	David Cox - Managing Director PO Box 7540, Christchurch, New Zealand
DairyNZ	John Williamson - Lead Research Technician Jane Lacy-Hulbert – Senior Scientist DairyNZ, Private Bag 3221, Hamilton, New Zealand

Product Information

SYGNET Anti-Bacterial Teat Wipes are packaged in a plastic dispenser containing 100 pre-moistened wipes (see Appendix). They are made from a non-woven tissue-like material and are impregnated with the ingredients listed below. The tissues are stored in a sealed container which dispenses individual wipes through a slot sealed with a cap (see Appendix).

Product Name: SYGNET ANTIBACTERIAL TEAT WIPES

Manufacturer's Product Code: CI 110

Uses: For surface control of *Escherichia coli, Staphylococcus spp., Lysteria spp., Salmonella spp., Campylobacter* spp. and other pathogenic bacteria.

Ingredients	**Cas No**	**Proportion**
Sodium salt of 4-chloro-3-methylphenol	15733-22-9	0.1%-0.2%
Isopropyl Alcohol	67-63-0	30%-40%
Sodium Lauryl Ethoxylate (27%)	68585-34-2	0.2%-2.0%
Citric Acid	5949-29-1	0.4%-0.8%
Water	7732-18-15	69%-57%

Health Effects:

Swallowed: May cause mild discomfort.

Eye: May be irritating to eyes.

Skin: Prolonged excessive exposure may cause mild skin irritation or dryness.

Experimental design

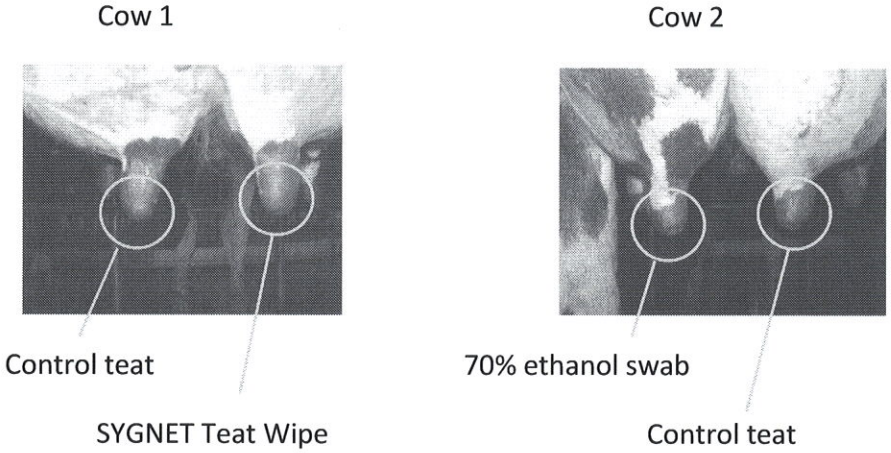
This work compared the efficacy of two disinfecting procedures in reducing the surface load of bacteria on cows' teats. Experiments were carried out using "natural exposure" conditions on cows from within the Lye Farm Research Herd. Natural exposure refers to the resident population of bacteria present on cows' teats arising from environmental sources and from skin colonisation.

A random selection of 40 cows from the Lye Farm Research Herd was used in this study, which ran during the month of October. All cows were milked twice daily throughout the trial, were grazed on pasture supplemented with grass silage when appropriate, and received post milking teat spraying with an iodine-based disinfectant following each milking. All testing was done at the morning milking prior to cups being attached. No cleaning or washing of teats was done prior to the wipes being tested. Only the left hind and right hind teats within cow were used. Cleaning treatments were allocated equally between left and right hind teats to avoid any experimental bias (see Figure 1).

The experimental disinfecting treatments were:

(a) The reference method (70% Ethanol-soaked cotton wool swabs)
(b) The test method (SYGNET Anti-Bacterial Teat Wipes)
(c) Control teat left uncleaned

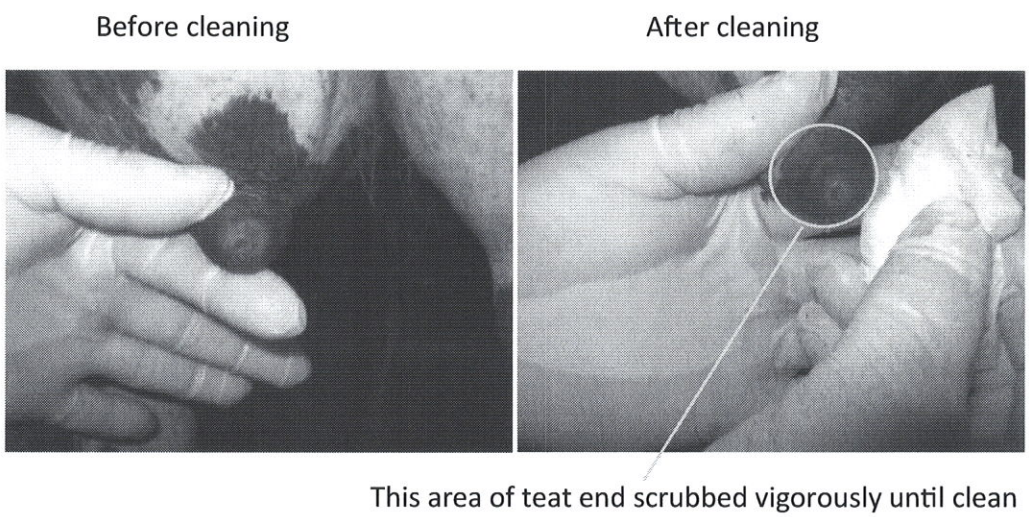
Figure 1: Allocation of treatments



Teat Cleaning Process

The teat cleaning process involved vigorously scrubbing teat-ends for approximately 10 seconds or until a new surface of a 70% ethanol soaked cotton-wool swab or SYGNET Teat Wipe remained clean (Figure 2). The area that was cleaned extended out to approximately 3 cm around the teat orifice. A period of approximately 1 minute was then allowed for the disinfecting process and drying to take place prior to teat-end swabbing. One teat (either left or right hind) remained unwashed and untouched to act as the control teat.

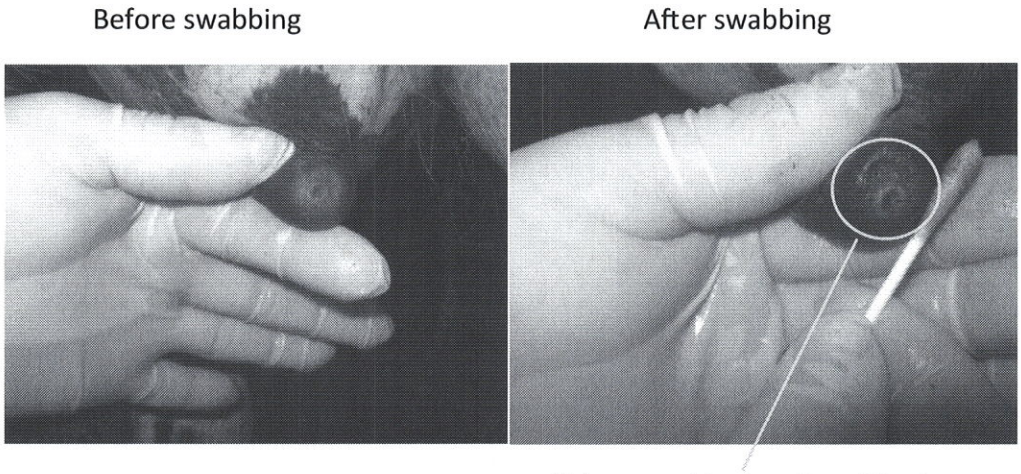
Figure 2: Teat end before and after cleaning with SYGNET Teat Wipe



Swabbing Process

Following cleaning, the teat end of both the treatment and control teats were swabbed (Lopez-Benavides 2009) using sterile individually packed swabs, pre-moistened with 0.1% peptone diluent. For swabbing a teat-end, the proximal third of the teat barrel was held between the thumb and fingers so that the teat-end could be easily manipulated for swabbing. The swab was removed from its plastic tube, and scrubbed vigorously across the teat end, whilst being rotated. Swabbing continued until such time as the teat-end looked visibly clean for the control teat (Figure 3). Following swabbing, the swab was placed back into its plastic tube, to prevent contamination, and transferred to the laboratory for bacteriological analysis. For consistency, all swabbing was carried out by the same operator.

Figure 3: Teat end of control teat before and after swabbing



Bacterial Counts

In the laboratory the swab tip was removed using sterile scissors and placed into a 1.5ml micro-centrifuge tube. A 1000 µl of 0.1% peptone diluent was added to the tube which was shaken vigorously for 30 s and then vortex mixed for 2 minutes to release the bacteria from the swab into the diluent. Following vortexing the swab tip was squeezed against the side of the tube to remove excess liquid. A 100 µl volume was then removed for serial dilutions into 0.1% peptone diluent.

Using methods similar to those described by Rendos (1975) and Hogan (1990), 10 fold dilutions ($1:10^1$, $1:10^2$, $1:10^3$ and $1:10^4$ were made and then 100 µl spread plated onto aesculin blood agar (for total bacterial count), Edwards Medium (for total streptococcal count) and MacConkey Agar (for total Gram-negative count). Plates were incubated aerobically at 37° C for 48 h prior to counting of viable bacteria. Bacterial counts were expressed as colony-forming units (cfu) per swab.

Statistical power calculation

This trial consisted of 2 parts:

(1) Comparing Treatments with Control: 70% ethanol soaked cotton wool swab or SYGNET Teat Wipes versus control (un-cleaned teats)
(2) Comparing Treatments: 70% ethanol soaked cotton wool swab versus SYGNET Teat Wipes.

The statistical power of the experiment was established to meet the requirements of part 2. The aim was to show bioequivalence of the SYGNET Teat Wipes with the reference method, 70% ethanol soaked swabs. To do this it was necessary to show that the confidence interval for the difference between the two treatments was no greater than a predetermined amount. The power calculation used 90% power and a significance level of 5%.

Using data from a previous experiment (Turner 2008), a simulation was run using estimates of the variance components to determine the effective standard deviation for the proposed design, with each cow having one of the rear teats untreated (i.e. not cleaned) and one of the two teat swabbing treatments on the other rear-teat. The estimated standard deviation from this simulation was 0.63.

The power calculation, using a predetermined limit for the difference between the two treatments of 0.6 on the $\log_{10}$ scale for bio-equivalence (i.e. no greater than a four-fold difference in bacterial counts), identified that 20 cows were required for each treatment, therefore the trial required 40 cows in total.

Results

Swabbing of teat ends showed differences in bacterial recovery between treatments. Counts were made of total bacteria, total streptococci and total Gram-negative bacteria. Log_{10} and actual numbers of bacteria recovered from swabs for control teats (uncleaned), 70% ethanol soaked swabs and SYGNET Teat Wipes are presented in Table 1.

Table 1: Treatment means for control (uncleaned teats), 70% ethanol soaked swabs and SYGNET Teat Wipes.

	Control	70% Ethanol	SYGNET	SED (tmt. vs. control)	SED (tmt vs. tmt)	P value (control)	P value (trt)
Log_{10} Total Bacteria	6.208	3.828	3.824	0.129	0.161	<0.001	0.980
Actual (cfu/swab) Total Bacteria	1,614,259	6630	6568				
Log_{10} Total Streptococci	3.884	2.091	2.044	0.207	0.247	<0.001	0.850
Actual (cfu/swab) Total Streptococci	7556	23	11				

Log_{10} Total Gram-negative	3.134	2.020	2.090	0.149	0.175	<0.001	0.692
Actual (cfu/swab) Total Gram-negative,	1261	5	23				

Log values were calculated after adding 100 to each value, to avoid zero counts. The log_{10} (count+100) data were analysed using mixed models with replicate as a random effect and treatment as a fixed effect. Data indicate that both treatments (70% ethanol swabs or SYGNET Teat Wipes) were significantly better than the control ($P < 0.001$), but not significantly different from each other ($P > 0.1$).

In all cases, uncleaned teats had significantly higher levels of bacteria present. Typically from uncleaned (control) teats, bacterial counts were in the range of log 6 (≥1,000,000 cfu/swab) for total bacteria, log 3.8 (≤10,000 cfu/swab) for total streptococci and log 2 (≥100 cfu/swab) for total Gram-negative bacteria. Large cow-to-cow variations for all bacterial counts were observed, depending on the degree of teat contamination with dirt or faecal material.

For both the 70% ethanol swabs and SYGNET Teat Wipes treatment groups, most swabs (≥70%) yielded no bacteria for total streptococcal and total Gram-negative bacterial types (Table 2). Some bacteria (mostly *Bacillus* spp., based on colony morphology on aesculin blood agar) were always isolated, but differences between treatments were not significant.

Table 2: The proportion of teat end swabs that yielded no bacteria for the 70% ethanol swab and SYGNET Teat Wipes treatments

Bacterial Group	% of swabs that yielded no bacteria	
	70% Ethanol swabs	SYGNET Teat Wipes
Total bacterial count	0[a]	0[a]
Total streptococcal count	75	75
Total Gram-negative count	95	70

[a] All swabs yielded some bacterial growth

It was observed during the cleaning process that often more than one wipe per teat was required to achieve the level of disinfection considered sufficient by the experimental operator.

Discussion

Large populations of bacteria including *Bacillus spp.*, streptococci, coliforms, other environmental bacteria and normal skin-related organisms are always present on the teat skin of cows grazed on pasture.

Before the insertion of intramammary antibiotic products, teat-ends must be cleaned to remove bacteria and thereby preventing infusion of extraneous bacteria into the udder of the treated cow. Teat disinfection should be seen as an important step when dry-cow (or other) intramammary antibiotics are being used.

This study was set up to evaluate the effectiveness of SYGNET Anti-Bacterial Teat Wipes compared to a reference method (70% ethanol soaked cotton-wool swab) for the disinfection of teat ends prior to the use of intramammary antibiotics. A random selection of cows teats (left and right hind only) which were naturally contaminated with environmental micro-flora were swabbed to establish levels of bacteria that remained on the teat end following cleaning.

Results from this study indicate that SYGNET Teat Wipes achieved a similar level of efficacy in terms of bacterial disinfection when compared to the 70% ethanol soaked cotton-wool swabs used as the reference method. Ethanol diluted to 70% with water was used as the standard in this study as it has long been recognised as a powerful disinfectant, effective against a wide range of microbes.

To achieve the level of bacterial reduction that was observed for both the 70% ethanol swabs and the SYGNET Teat Wipes the teat-end needed to be vigorously scrubbed until it appeared visibly clean. For the SYGNET Teat Wipes this required at least one wipe per teat depending on the level of contamination. During this study it was clear that one wipe per cow was insufficient to provide adequate cleaning. The packs provided contain 100 wipes which is, therefore, sufficient for 25 cows if one wipe per teat is used.

The timing of this study, which coincided with wetter spring conditions, may have meant that teats were more heavily contaminated with bacteria and organic material than may be the case when using intramammary treatments on cows at dry-off, when in New Zealand teats are typically less contaminated.

Conclusion

Data from this study suggest that SYGNET Teat Wipes can achieve a comparable level of teat disinfection when compared to 70% ethanol soaked swabs used as the reference standard, with no significant difference in the numbers of bacteria recovered between the two treatments. Both disinfecting treatments showed substantial reductions in numbers of bacteria recovered compared to uncleaned teats.

The physical strength of the wipes during teat scrubbing and their convenience of use were positive features.

There are some improvements that could be made to the canister that the wipes are packaged in. It was noted that the cap did not always fit firmly, and if left uncapped these types of swabs tend to dry out and lose their effectiveness due to the evaporation of the alcohol.

Some improvement could also be made to the label format in terms of more specific instructions on how to use the wipes. It needs to be stressed that vigorous scrubbing is required to achieve sufficient cleaning of the teat end, and at least one wipe per teat is required.

References

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