Blowfly resistance. The “Nimmitabel strain” and its implications for fly control

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Nimmitabel strain

- History of resistance *Lucilia cuprina* Australian Sheep Blowfly
  - What resistance really looks like.
- Nimmitabel
  - Field history
  - Laboratory follow up
    - EMAI
    - Yarrandoo
  - Field Study
  - Larval Implant Study

- Current recommendations.

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Where is Nimmitabel?

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Blow fly resistance

- *Lucilia cuprina* has rapidly developed resistance to all the predominant fly strike chemicals within 5-6 years of introduction with the exception of cyromazine (Vetrazin®) and dicyclanil (CLiK®)
- Why? *L cuprina*
  - Biological potential (reproductive ability)
  - a strict almost obligate parasite
  - Our intolerance of fly strike
  - Genetic diversity in the fly and other factors?

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Fly resistance

- Organochlorines - DDT, Dieldren
  - Fly strike protection completely lost 1958
- Organophosphates - Diazinon, malathion
  - Fly strike protection reduced
    - 12 weeks to only 2-4 weeks
  - Extensive use and selection 98% of blowflies resistant by 1970
  - Main fly chemical until Vetrazin (1979)
- Benzoylphenyl ureas (BPUs) – diflubenzuron
  - 1993 released - 2001 resistance reported and
  - 2006 Tara property no protection
  - 2008 all products fly claim removed

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What do these products have in common?

- All were used for both fly and lice.
  - Tara strain were used for 4 consecutive lice and fly treatments over 2 years before resistance developed.
- “Cross resistance” existed to all 3 chemicals
  1. from completely different chemical groups
  2. different modes of action

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VETRAZIN Spray-On ®

- 6% cyromazine
- provides up to 11 weeks protection
- quick and easy to apply
- minimum 6 weeks wool
- 2 month wool withholding period

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CLiK® Spray-on

- 5% dicyclanil
- Water-based spray-on
- Only product registered for season-long protection 18-24 weeks
- No field resistance detected
- Any length wool (3 month withhold)
- 120 day ESI
- Rain-Lock Technology

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CLiKZiN® Spray-on

- 1.25% dicyclanil
- Water-based spray-on
- up to 11 weeks protection
- 1 month wool withhold
- 3 weeks ESI
- 7 day meat WHP

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CLiK® in Unmulesed sheep

- Three published studies looking at efficacy of CLiK® in unmulesed sheep.
- Larsen, J., Tyrell, L. and Anderson, N. (2012), Prevalence of breech-strike in mulesed, clipped and unmulesed Merino hoggets in south-eastern Australia. Australian Veterinary Journal, 90: 158–166.
- Hosking BC, George AG, Efficacy of CLiK® Spray-On in Preventing Naturally Occuring Blowfly Strike When Applied to Unmulesed Sheep *Intern J Appl Res Vet Med • Vol. 7, No. 1 & 2, 2009*
- James, P., Cramp, A., Winkleman, J., Mcphie, R. and Brown, G. (2009), Strategic use of crutching and dicyclanil to protect unmulesed sheep against breech strike. Australian Veterinary Journal, 87: 138–141.

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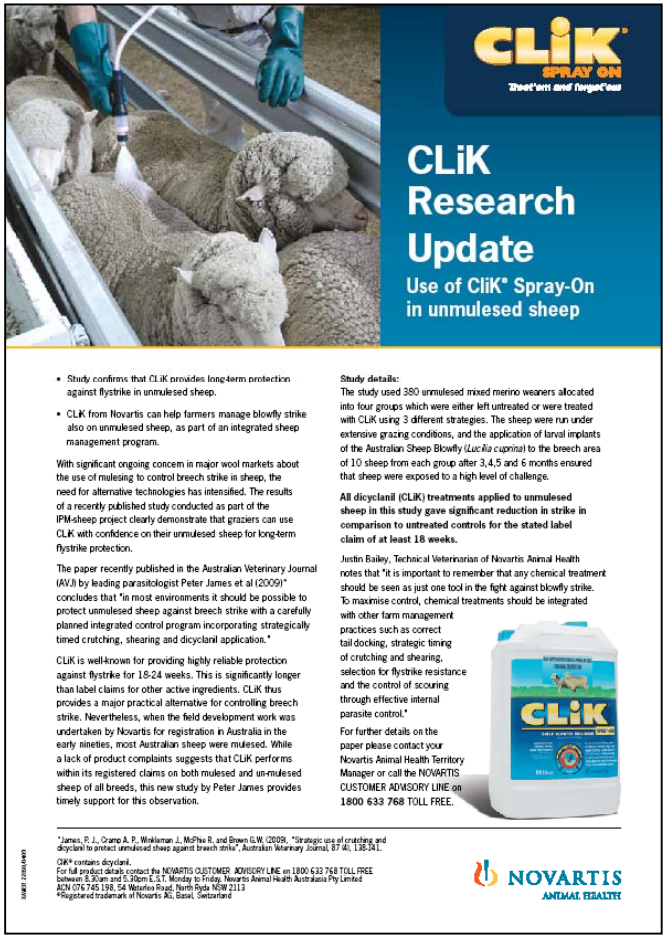
CLiK® – Studies in un-mulesed sheep

- Registration studies conducted in mulesed sheep
- Independent studies in VIC & NSW – 10 farms/ 3028 sheep
- Good fly pressure (moderate to high)
- Wide range of sheep type, breeds, ages, climates & treatment timing
- CLiK® in un-mulesed sheep as effective as mulesed sheep (0.23% @ 28 weeks)



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CLiK ® – Studies in un-mulesed sheep

- 380 mixed sex, un-mulesed, Merino weaners (most susceptible group) at Longreach
- Sheep were made susceptible and 100 larval implants applied
- All treated groups were significantly different from the control group at 3 & 4 mths (18 weeks)
- CLiK in un-mulesed as effective as mulesed

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PRODUCTION ANIMALS

Strategic use of crutching and dicyclanil to protect unmulesed sheep against breech strike

RJ James,* AP Cramp,† J Winkelman,‡ B Mcphie‡ and GW Brown‡

Objective To test strategies for the application of dicyclanil and mid-season crutching to maximise protection of unmulesed sheep against breech strike.

Procedure Three hundred and eighty unmulesed Merino weaners were randomly allocated to four groups either left untreated or treated by different strategies with 50 g/L dicyclanil. Treatments included breech treatment alone and breech plus body treatment, with two application times, immediately after shearing and 6 weeks after crutching or shearing. To assess protection, larval implants with newly hatched *Lucilia cuprina* larvae were applied to 10 different sheep from each group at 3, 4, 5 and 6 months after crutching and shearing and assessed for the development of strike at 48 hours. The concentration of dicyclanil was measured in wool samples clipped from the breeches of the test sheep.

Results All dicyclanil treatments gave significant reduction in strike in comparison to controls up until 4 months after crutching but protection in the sheep treated immediately after shearing had waned at 5 months. Treating at 6 weeks after crutching provided significant reduction ($P < 0.05$) in strike for 6 months. Results for strike incidence immediately after shearing and concentration of dicyclanil in the breech wool also suggested improvements in protection by delaying treatment for 6 weeks.

Conclusion In most environments it should be possible to protect unmulesed sheep against breech strike with a carefully planned integrated control program incorporating strategically timed crutching, shearing and dicyclanil application. Delaying treatment with dicyclanil to at least 6 weeks after shearing or crutching increased the protection provided in comparison to treatment immediately after shearing.

Keywords fly strike; mulesing; dicyclanil; *Lucilia cuprina*; sheep

Blowfly strike, caused primarily by *Lucilia cuprina* (Wiedemann) is one of the major health problems of the sheep industry in Australia.[1] Breech strike comprises the majority of strike in most years.[2] Mulesing has been the keystone procedure in integrated programs for flystrike control over many years and is extremely effective in preventing breech strike in both Merino sheep and plainer bodied breeds,[3] but there has been heightened effort to find new methods of controlling breech strike because of animal welfare concerns.[4] An intense research effort is underway to find alternative to mulesing and it is expected that replacement technologies will be available by 2010.[5] However, many wool growers are seeking the best ways of managing breech strike in unmulesed sheep with currently available technologies.

Assessing the effectiveness of fly control strategies under field conditions is difficult because of the requirement for field challenge and because of ethical considerations in leaving sheep unprotected against flystrike during periods of high fly activity. This study used larval implants with first instar *L. cuprina* on sheep run under extensive grazing conditions to assess the period of protection provided by control strategies based on mid-season crutching (removal of wool from upper hindlegs, perineal region and base of tail) and the application of dicyclanil. Using larval implants had the advantage that a high level of challenge could be assured, the development of strikes could be closely monitored and strikes could be terminated early to minimise stress on the experimental sheep.

Materials and methods

Sheep and location

The study was conducted with 380 unmulesed ewe and wether Merino weaners, crutched in December 2005, shorn in June 2006 and run under extensive grazing conditions at Rosebank Research Station at Longreach in western Queensland. Longreach has an average annual rainfall of 432 mm and mean winter and summer maximum temperatures of 23.9°C and 37.3°C, respectively. Rainfall during the study was 371 mm, with 303 mm received between January and April. The sheep were managed as a single commercial flock in a 400 ha paddock according to the Australian Model Code of Practice for the Welfare of Animals.[6]

Treatment

The weaners were allocated to four treatment groups, balanced for sex, after crutching in December. The treatments were: (a) untreated control; (b) treated on the breech immediately after crutching with 10 mL of 50 g/L dicyclanil (CLiK®, Novartis Animal Health Australasia Pty Ltd, North Ryde, NSW); (c) body and breech treatment immediately after crutching with 50 mL of 50 g/L dicyclanil formulation in three strips, two along either side of the backline and one over the tail, and (d) as for (b) but treated 6 weeks after crutching. All sheep were shorn in June and the treatments supplied as described previously except that for group (c) the sheep were treated at 6 weeks after shearing, rather than immediately after shearing. All treatments were applied from the manufacturer-supplied hand held spray-on gun strictly according to the manufacturer's instructions.

Larval implants

Larval implants were applied to the breaches of 10 different sheep selected from each group of 95 at 3, 4 and 5 months after crutching and at 3, 4, 5 and 6 months after shearing. Sheep were not re-used within the two 6-month periods. As protection had clearly broken

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Cyromazine

- No resistance seen
  - 1979 until today
- Why?
  - Not used for lice?
    - Dual use of chemicals in short and long wool probably contributed to resistance to OC's, OP's and BPU's
  - Clues from laboratory induced resistant flies
    - Resistant flies less fit or robust or lethal gene
    - Genetically incomplete dominance
    - Narrow selection window
    - Resistance offered little advantage over fully susceptible flies.

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What is resistance?

- International Resistance Action Committee (IRAC) define insect resistance as

"*a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species*."

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History of Nimmitabel strain

What happened on the farm and why were these maggots tested for resistance?

A mob of unmulesed ewe lambs were struck 3-4 weeks after application of a generic Cyromazine spray-on (CSO).

- These lambs were treated on the breech and the body but were only struck on the breech.
  - concerns about the amount and application pattern of the product on the breech.
- Product was re-applied
- Maggots were collected as a precaution and sent to Dr Garry Levot's lab at EMAI, NSW DPI.

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Were there any other failures of flystrike preventative products on the property?

Wether lambs and adult ewes were treated with CLiK® which performed as expected despite very heavy fly pressure and significant rainfall events.

The ewe lambs were only struck on the breech and had no body strike and critically, Cyromazine Spray On worked as expected on both the body and the breech after it was reapplied.

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Research/stewardship questions

1. Will the Nimmitabel strain affect the protective period of Novartis products?
2. Will the strain persist on farm?

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Larval Implant studies

- Larval implant studies
  - an implant study was conducted using sheep treated with either Vetrazin® SO or CLiK® SO and then implanted with larvae from; the Nimmitabel strain or a triazine “susceptible” reference strain.
- Both the Nimmitabel strain and the reference strain performed as expected and there was no difference between strains
  - CLiK® 18 weeks (trial terminated)
  - Vetrazin® SO 13 weeks (strike established at 14 weeks)
- We expect no difference between the Nimmitabel strain as far as protective period for either Vetrazin® (we can’t conclude anything about generic cyromazines from this study) or CLiK®

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Field studies 2011/12

- Original “Nimmitabel” property
- 385 lambs treated with Vetrazin® Spray On and 198 lambs treated with CLiK®, December 2011
- Continually monitored by producer and once every 4 weeks Novartis for any strike
- High fly challenge trial
- Fly Pressure-
  - monitored by fly traps
  - heavy fly pressure for most of trial
- Rainfall-
  - Significant rain in Dec-April (405 mLs)
- No strike in either group at 14 weeks
- At 18 weeks, 5 lambs with breech strike Vetrazin® SO
- No strikes in CLiK® group

Field studies 2011/12

- Results
- No strike established in either group at 14 weeks
- At 18 weeks,
  - 5 lambs with breech strike Vetrazin® SO (registered protective period 11 weeks)
- No strikes in CLiK® group

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Conclusions

- These studies demonstrated that, against this field isolate, under both field and controlled laboratory conditions, Vetrazin® spray–on and CLiK® each maintained the registered protective period after treatment
- No sheep treated with CLiK ® were struck within registered protective period.
- Clearly shows the importance of application to protective period
  - Protective Period = Active + Formulation + Application[2]

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Did the strain persist on farm?

- Why would this occur?
  - Resistant lab strains of *Lucilia cuprina* and *Musca domestica* (house fly) tend to die out of populations over a number of generations.
- Any evidence of it dying out.
  - The evidence is inconclusive
    - One larvae found of several 1000 cultured 11/12 (3-4% 2010)
    - possible reduction in the % of resistant strains in the population but the data is inconclusive at this stage.
- Survey of Neighbouring properties 2011/12
  - Found in low numbers in 3 out of 5 properties
    - No control failures in the area/district as measured by product complaints?

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Recommendations

"No need to change your current practice most importantly do it once and do it right"

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Current Flyboss recommendations

- Use Integrated Pest Management (IPM)
  - chemical and non chemical means of making sheep less attractive to fly
- Use a different chemical to treat fly struck sheep to the one you used to prevent fly strike (different mode of action) this breaks helps break cycle
- Use different chemicals for treatment of fly and lice

"Do it once and do it right"

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Conclusion

- Resistance to cyromazine has been detected at the laboratory level in vitro tests
- It is a mild resistance and has not caused any failure in the field of cyromazine based products
- Field observations, trials and laboratory experiments suggest that we expect no reduction in protective period for CliK ® or Vetrazin ®
- Producers should use fly products that suit their management in a sustainable manner, that maximises production and animal welfare.

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Conclusion

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- Acknowledgements
  - The producers on the original Nimmitabel property
  - Dr Garry Levot EMAI cooperation and supply of Nimmitabel strain larvae

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