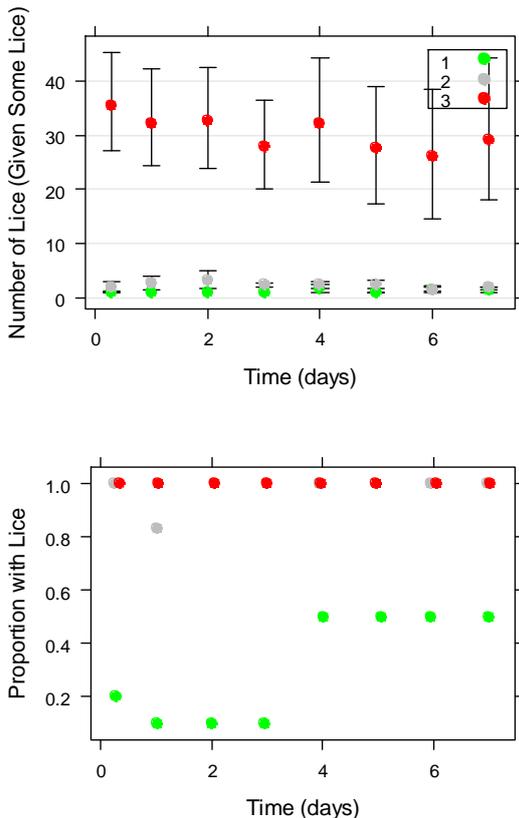


INFESTATION DETERRENT EFFECT OF X92001483 AGAINST ISCHNOCERA LICE ON POULTRY

Title	
Investigator	Jennifer Ketzis, PhD
Sponsor	Oystershell Laboratories
Objective	Assess the effect of X92001483 (Elimax shampoo) in discouraging the reinfestation of Ischnocera lice on poultry over 7 days
Study Design	<p>The aim of this study was to evaluate the effect of X92001483 in discouraging the reinfestation of Ischnocera lice on poultry over 7 days.</p> <p>All of the chickens were naturally infested with lice, 33 chickens were included in the study and divided into the following treatment groups:</p> <p>Group 1 (test group): 10 <i>chickens without lice and treated with X92001483</i></p> <p>Group 2 (control group): 6 <i>chickens without lice and not treated; used to assess how quickly lice repopulate a louse free chicken</i></p> <p>Group 3 (seeder group): 17 <i>lousy chickens; used as a reservoir for the lice</i></p> <p>The chickens (adult layers) with natural lice infestations were randomly assigned to the groups. All lice counts were conducted by a person blinded to the treatments.</p> <p>The general design was based on the WAAVP guidelines for assessing persistent activity of products for lice on cattle.</p> <p>Study Day -3 On arrival (Study Day -3 (SD -3)), the chickens were transferred to the animal unit and placed in a pen approximately 4 x 8 ft.</p> <p>Study Day -2: Six (6) chickens, randomly selected from the 33 chickens, were treated with E004490 (the licidal portion of X92001483) (see treatments) to kill the lice. Approximately 15 min after treatment, the chickens were washed (with Dawn dishwashing liquid), towel dried and gently air dried with a blow drier. After the chickens were dry, they were housed in a pen approximately 4.5 x 4ft. There was no physical contact between these chickens and the remaining 27 chickens until SD 0.</p> <p>Study Day 0: The lice on 5 areas (approx 2 x 2 cm) of each chicken were counted and recorded.</p> <p>Ten of the 27 lousy chickens, randomly selected, were treated with X92001483 (see treatments) and dried as previously described. The 6 previously deloused chickens were rinsed with water and dried.</p> <p>All 33 chickens were then housed in a pen approximately 4 x 8 ft.</p> <p>Lice were counted on 5 areas of each chicken at +8 h post-treatment \pm 18 min (see deviations).</p> <p>Study Days 1 to 7: Lice were counted on 5 areas of each chicken at +24 h, +48 h, +72 h, +96 h, +120 h, +148 h and +172 h \pm 1 h post-treatment (see deviations).</p> <p>Study termination of animal phase:</p>

	The study terminated after the +172 h count.
Animals	Thirty four adult layers (white leg horn crosses Rhode island red crosses) were acquired for the study. Thirty three, identified by leg band (e.g., cable tie), were deemed healthy by a veterinarian to participate in the study. All veterinary findings from SD -3 are listed in Table 1. In addition to these findings, chicken 86 (Group 3) appeared lethargic with an enlarged ventriculous on SD -2. However, upon later examination, the chicken appeared healthy. It is believed that there might have been difficulties with laying an egg and once the egg was laid, the chicken was fine.
Treatments	<p>The treatment procedure used was as per the proposed instructions for use on humans. Efficacy of the lice removal was determined via visual examination of each chicken.</p> <p>Group 1 (SD 0) chickens were treated with X92001483. The product was sprayed on to each chicken until the chicken was considered fully covered. The feathers were moved backwards and the skin and feathers sprayed with the product. After 15 min, a small amount of water was applied to the chicken to allow the soaping agent to react. After the product began to feel like soap, the chicken was completely rinsed. The chickens were then towel dried and blow dried using cool air.</p> <p>Group 2 (SD -2) chickens were treated with E004490. The product was sprayed on to each chicken until the chicken was considered fully covered. After 15 min, the product was removed by washing the chicken with Dawn brand dish detergent. The chickens were then dried as described for Group 1.</p> <p>Group 2 (SD 0) chickens were rinsed with water. The chickens were then dried as described above.</p>
Assessment of effectiveness	<p>Less than 2 h pretreatment, the number of lice in five approximately 2 cm x 2 cm areas was counted on each chicken. The areas included the ventral and dorsal surface of each wing, the breast, the tail feathers and the back.</p> <p>At +8 h post-treatment \pm 18 min and +24 h, +48 h, +72 h, +96 h, +120 h, +148 h and +172 h \pm 1 h post-treatment (see deviations), lice were counted on the 5 designated areas.</p>
Results	Groups 1 and 3 were not statistically different in regards to their lice counts pretreatment (Kruskal-Wallis test; $p = 0.0688$; 0.687 adjusted for ties). Throughout the study post-treatment, the number of lice on the chickens in Groups 1 and 2 were significantly different from Group 3 (Kruskal-Wallis test; $p > 0.05$). When Groups 1 and 2 were compared, they were significantly different at all time points post treatment except +148 h ($p > 0.05$; Kruskal-Wallis test; see table 4). However, when the number of infested chickens was compared (Fisher's Exact), Groups 1 and 2 ceased to be significantly different at +96 h. These results are represented in the graphs below.

	 <p>(Top) Average (\pmSD) number of lice for lousy chickens. (Bottom) Proportion of lousy chickens. (1) group 1 treated with X92001483, (2) group 2 pre-treated with E004490, and (3) group of lousy chickens.</p>
<p>Discussion</p>	<p>All of the chickens had heavy lice infestations, with lice on the wings, feathers and body.</p> <p>During product application, the lice tended to move away from the area of application (Group 1). During product application (Groups 1 and 2), the lice stopped moving. That is, the effect of the product could be observed during application.</p> <p>During product removal (after 15 min; Groups 1 and 2), dead lice could be seen on the chickens. Most of these lice came off of the chickens during the washing. However, some did remain on the chickens.</p> <p>X92001483 washed off of the chickens relatively easily. With the addition of a small amount of water, the product formed a soap that washed off. However, all parts of the chicken had to be carefully dampened with water to form the soap. The Group 1 chickens were very clean after the process and remained so for much of the study making the maintenance of blinding difficult.</p> <p>Group 2, treated with E004490, were left with a slight oily film. This oily film remained through SD 1, also making the maintenance of blinding difficult.</p> <p>All of the chickens grouped together when group housed. That is, none of the chickens were avoided by the other chickens. Therefore, there was sufficient opportunity for lice to transfer from chicken to chicken.</p>

	<p>The rate at which the lice repopulated the Group 2 chickens was likely a reflection of the natural rate at which lice are transferred between chickens. The overall reinfestation level remained low during the study, but the chickens did consistently have lice.</p> <p>Many of the lice seen on the Group 1 chickens did not run from exposure to light and move toward the base of the feathers and skin surface as is common with these lice. This might have been due to the deterrent properties of the product.</p> <p>By 96 hours post treatment, the chickens in Group 1 appeared to be able to maintain a lice population. That is, the deterrent properties no longer appeared to be effective.</p> <p>In this study, both products were effective in killing the lice on the chickens. Lice did begin to repopulate the chickens treated with E004490 within 8 hours of group housing. While two chickens treated with X92001483 had lice 8 hours post treatment, X92001483 (Elimax shampoo) provided significant protection from reinfestation for 72 hours.</p>
References	<p>USDA. 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. Holdsworth PA, Vercruysse J, Rehbein S, Peter RJ, Letonja T, Green P. 2006.</p> <p>World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of ectoparasitocides against biting lice, sucking lice and sheep keds on ruminants. Vet Parasitol 136(1):45-54.</p>