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EVALUATION OF 4 PHOSPHOROTHIOATED SYSTEMIC INSECTICIDES AS VAMPIRICIDAL AGENTS (Preliminary Results).

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Vampire Bat Control Project

Paper presented at the 8th. Annual Reunion of the National Institute for Livestock Research, January 26-29, 1971.

When we initiated the design for systems of controlling vampire bats, (Desmodus rotundus) vector of bovine paralytic rabies, we took into account the experiences of ecologists and found the following problems which limits control efforts at the roost.

1) the vampire bat lives together with other species which are beneficial for agriculture and biological balance

2) From the economic and practical point of view, it is impossible to locate and destroy all vampire niches.

3) if we destroy a small number of refuges we would probably cause migrations to unknown places, making the control more difficult.

Therefore, our objective was to evaluate specific control methods for Desmodus rotundus which will not alter the biological balance.

One of these methods may be the application of chemical substances in or on bovines so that vampires could take them from the skin or blood of cattle.

We analyzed in vitro the power of four phosphorothioated compounds as vampiricidal agents which are used as systemic insecticides in bovines and ovines. Two,

Warbex and Cygon, are from American Cyanamid, and the other two, Ronnel and Ruelene, are from Dow Chemical.

The search for known chemical compounds was initiated because:

1) it saves time and money to work with substances whose pharmacological and toxicological effects are known.

2) the experience of other investigators on the hazards these compounds might cause to domestic species will guide us in applying them.

According to Radeleff these compounds work at the nerve endings producing an intoxication similar to that of an overproduction of acetylcholine.

The nerve impulse is coordinated by two substances, acetylcholine and cholinesterase. The former causes a change in the permeability of the membranes that separate the termination of the axon from the sole plasm of the muscle cell. The latter inactivates the acetylcholine, and the impulse is suspended, which allows a refractory period during which the effector organ rests and the axon is repolarized.

When the phosphorothioated compounds are in action, they release, by an esterase action, their phosphorous radical which reacts with acetylcholinesterase. This the forms a stable compound. The acetylcholinesterase loses its capacity to break the acetylcholine molecule which permits a continual flow of electrons causing severe disfunctions or death.

With this knowledge, and before initiating the evaluation of the drugs, we looked for bases to suppose that an nontoxic dose for cattle would kill vampires. Unfortunately we still don't know enough about Desmodus rotundus so the information was obtained indirectly.

There is an insectivorous bat, Eptesicus fuscus

pallidus, whose average cardiac rate (Eugene Studier et al, 19..) is 447/minute. We used the information from this bat because it is close to Desmodus rotundus in the phylogenetic scale.

The cardiac rate of bovines is 60/minute, and the frequency of the motor impulses in domestic animals is 10-90/second (Dukes 19..) but in other vertebrate species it may be more than 1,000.

We made a correlation of cardiac frequencies and found that the bat's is about 7.5 times higher than the bovine's. Therefore, the frequency of nerve impulses is higher. Since each nerve impulse passes by a reaction of acetylcholinescholinesterase, this reaction will be produced many more times in bats and if the cholinesterase is inactivated and acetylcholine goes on being produced, its accumulation at the nerve terminations will be greater and the toxic effects will appear sooner and be more acute. We can expect then, that a nontoxic dose for bovines could be fatal to bats.

We initiated our LD_{50} study of these compounds following the Thompson and Weil method, modified in 1952, except we diluted the compound in defibrinated cattle blood instead of corn oil.

We used two groups for each drug, and each group had 8 individuals of the same sex. In the female group we used only non-pregnant individuals. Each group was divided in 4 sub-groups of two animals. Each sub-group was dosed with the compound at a 50% higher level than the sub-group immediately below.

The bats were fastened 24 hrs and then we introduced the dose using a rigid gavage.

After that, the bats were observed for a 14-day period. They were fed with defibrinated cattle blood, and the colony was maintained with a temperature of 21-23°C and a relative humidity of 40-60%.

During the observation period each dead was recorded. For those animals which died the first day we performed a necropsy to find if the lungs were full of blood. This precaution was taken to detect possible errors in gavaging. If this occurred, new animals were used, and the dose level was repeated. Whenever it was necessary to increase or decrease the dose levels to adjust the number of dead individuals to the calculating tables, we followed the same method.

Results obtained wese as follows:

We calculated two toxicity factors for each compound, LD_{50} and 95% confidence limits.

The first one is the amount of a toxic agent necessary to kill 50% of a group of animals under study in a determined period; 14 days in this case. The second one is the dose able to kill 95% of animals and is found between two levels; a maximum and a minimum.

Warbex LD_{50} for males was 8.06 mg/kg with 18.57-3.67 mg/kg, 95% confidence limits. LD_{50} for females was 2.4 mg/kg with 1.06-5.40 mg/kg, 95% confidence limits.

Results obtained from Cygon wese as follows: LD_{50} for males was 6.80 mg/kg (5.30-3.02 mg/kg, 95% confidence limits). LD_{50} for females was 12.38 mg/kg (50.40-3.03 mg/kg, 95% confidence limits).

We used Ronnel at 18 mg/kg at the lowest dose level and at 94 mg/kg at the highest. Maximum doses for Ruelene were 62 mg/kg. In both drugs the same level was used for males and females.

Conclusions:

We do not expect Ronnel and Ruelene be effective, because the toxic dose for cattle is 100-125 mg/kg and the highest level for the 95% confidence limits exceeds the safety limits.

Before we analyze the results obtained, I would like to make it clear that, though Cygon is a well known product, it is not registered and we willpproceed with caution.

(Graph of Results of Cygon and Warbex)

In this graph we see on the vertical line milligrams from 0 to 50, and on the horizontal line we see the results obtained in the experiment and the recommended doses for bovines.

The recommended dose of Cygon for cattle is 10 mg/kg administered orally during 10 days. The LD_{50} for males was 6.80 mg/kg, which means we have an effectiveness margin of 3.20 mg/kg, which represents a vampiricidal power of 68%. Nevertheless, we cannot expect a higher percentage because the 95% confidence limits' highest level is 15.30 mg/kg which is 5.30 mg/kg in excess.

LD_{50} for females was 12.38, which is 2.38 mg/kg in excess and the highest level at 50.40 mg/kg. Therefore its vampiricidal power may be low. The wide variation in toxicity of the drug was shown in the mortality rate obtained; only 3 out of 8 individuals died.

Nevertheless, we have great hopes for Warbex. The dose recommended by Radeleff is 10 mg/kg for 10 days in a food additive. The minimum toxid dose for cattle is a little higher than 50 mg/kg. LD_{50} of Warbex for male vampires is 8.25 mg/kg which gives us an effectiveness margin of 1.75 mg/kg each day. The maximum limit of the 95% confidence limits is 18.57 mg/kg, so we have 8.57 mg/kg in excess and we can expect 60% effectiveness.

LD_{50} for females was 2.4 mg/kg which gives a margin of 7.6 mg/kg. The highest level of 95% confidence limits is 5.4 mg/kg, that means that we still have a margin of 4.6 mg/kg. So, we can expect that this drug will be more effective than the others.

Above, we have analyzed the compounds using only one application. Nevertheless these drugs have accumulative

properties and we can apply it during over a 10 day period without troubles to cattle. Considering previous experiences, that vampires usually return to feed on the same herd, we can expect that both drugs would be effective in a field test.

There are still two more possibilities for the use of Warbex. First, the minimum toxic dose for cattle is more than 50 mg/kg. That means we have a margin of 40 mg/kg, and if it were necessary we would use higher doses, considering of course, all precautions to avoid long term intoxication. Secondly, these compounds, even though they do not cause death directly, produce alterations in equilibrium, movement incoordination, diahrrea, renal and cardio-respiratory alterations. An animal so affected could be an easy victim of adverse conditions and would probably die in the field.

Encouraged with these results we will continue evaluating Warbex next month, applying it systemically on cattle.

I want to emphasise that we are still in the experimental phase and have a long way to go before we recommend this as a control method. This will occur when we are absolute certain Warbex will be effective, practical and economic, and that it will not damage either cattle or man.

Thanks!

GRAPH OF RESULTS OF CYGON AND WARBEX

Drug	LD_{50}	95% Confidence limits
Warbex:		
male	8.06 mg/kg	18.57-3.67 mg/kg
female	2.40 mg/kg	5.40-1.06 mg/kg
Cygon:		
male	6.80 mg/kg	15.30-3.02 mg/kg
female	12.38 mg/kg	50.40-3.03 mg/kg

	Maximum level
Ronnel:	
both sexes	94.0 mg/kg
Ruelene:	
both sexes	62.0 mg/kg