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STUDIES ON "KASEN" OF HORSES IN HOKKAIDO VII.

APPLICATION OF REPELLENTS AGAINST "KASEN" IN 1958 AND 1959

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INTRODUCTION

On the basis of the results obtained from the writers' several investigations\(^{13}\) on the general subjects of the therapeutic treatment on "kasen", it was recognized that the most effective therapy included the use of antihistamine preparations in venous injections and sufficient spraying of insecticides (DDT and BHC) during summer season.

In order to prevent an attack of insects upon animals, recently, application of various type repellents other than insecticides were conducted by many workers. When applying the repellents for animals, an insect is repelled by the medicine, so, the animals are free from an attack of the insect. Investigation of repellents for insects have been conducted since several years ago, and several thousand medicaments against mosquitoes have been examined\(^{16}\). In the early 1940's, vegetable and mineral oils\(^{6,15,17}\) were mainly examined as repellents against mosquitoes, but effectiveness was limited to only 20~40 minutes. So, practical use was not conducted against insects. In the mid-1940's, dimethyl phthalate, indalone, rutgers 612 and formula 6:2:2 medicine etc. were examined\(^{11,12}\). These medicines proved more effective against as repellents than the oil repellents. From late 1940's to the early 1950's, many pharmacological preparations were found\(^{18,20}\), which show effective repellency against insects. Further, recently, it was recognized that pyrethrum has also repellency against insects in addition to the value as an insecticide. So, pyrethrum was applied with some insecticide as repellent. Furthermore, investigations of synergistic medicine were also carried out. Piperonyl butoxide is a common synergist, which effectively increases repellency when used with a repellent, but piperonyl
TABLE 1. Experimental Methods and Results in 1958

<table>
<thead>
<tr>
<th>GROUP MEDICINES</th>
<th>CASE SPRAYING PERIOD</th>
<th>ITCHINESS</th>
<th>CHANGES OF LESIONS IN THE SKIN</th>
<th>CHANGES OF EOS. CELL COUNTS</th>
<th>RESULTS</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I R1</td>
<td>1</td>
<td>++ ++</td>
<td>- ++</td>
<td>Increase</td>
<td>Fell ill</td>
<td>R1 was not sprayed sufficiently</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+ + +</td>
<td>+ - -</td>
<td>Decrease</td>
<td>Fell ill</td>
<td>Sprayed slightly</td>
</tr>
<tr>
<td>II R2</td>
<td>3</td>
<td>- ++ -</td>
<td>+ + +</td>
<td>Increase</td>
<td>Fell ill</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>- ++ +</td>
<td>+ + +</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>III R4</td>
<td>5</td>
<td>+ - -</td>
<td>+ - -</td>
<td>No change</td>
<td>Fell ill later recovered</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>+ - -</td>
<td>+ - -</td>
<td>Increase in the B</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>IV R6</td>
<td>7</td>
<td>- - -</td>
<td>- - -</td>
<td>No change</td>
<td>Completely prevented</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>- - -</td>
<td>- - -</td>
<td>Decrease</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Notes: A...Initial stage of the observation (May & June)
     B...Middle stage of the observation (July & August)
     C...Terminal stage of the observation (September & October)
butoxide itself has no value as a repellent to insects.

The authors made an experiment in 1958 to find some protection against horses being attacked by "kasen" in the summer season. They applied 4 different kinds of medicine named R₁, R₂, R₄ and R₆ which were sprayed in summer season. It was found that the cases on which R₆ was used did not show the symptoms of "kasen" through the whole course. Further in 1959, the authors also conducted the same examination as in 1958 employing only R₆. The conclusion is that R₆ is satisfactorily effective (preventative) against the disease.

I. TESTS IN 1958

1. Materials and Methods

General view of experimental materials and methods is given in table 1.

1) Locality of experiment

The experiment was made on patients living in Kotoni district near Sapporo; the fields were preponderantly paddy. The blood sucking insects occur in summer season in these fields and a majority of horses living in this area suffered from attacks of insects and "kasen" disease.

2) Experimental horses

Eight animals were examined for the experiment. They were 6 to 12-year-old and all well nourished. Hair color was black in 6 cases and black-brown in 2 cases. Almost all the cases were female mongrel Percheron; they were engaged in farm work in the daytime every day and were fed in stable. The patients had all been attacked by the disease every summer; all of them had had "kasen" previously. During the course of the experiment, the animals were given mainly pasture grass and sometimes green hay by the owners.

3) Repellents and experimental methods

Four kinds of medicaments, as shown in table 2, were tested in the present experiment. The medicines were named as R₁, R₂, R₄ and R₆, respectively, "R" being the initial letter of repellent. The concentrations shown in table 2 are those of the original solutions. When using the medicines, the respective solutions were diluted 1:20 with water and sprayed with a compressed air hand sprayer. The medicine was sprayed by the owner upon the body surface of the patient mainly the neck and the tail, and on the outside and inside walls of the stable. Eight experimental animals were divided into 4 groups of 2 horses each. Spraying period was middle of May to last week of October; the medicines were sprayed daily during that time.

Hematological examinations and clinical observations were conducted at 2 weeks intervals during the spraying period.

In order to make clear the results, the course of observations was divided into 3 stages: initial stage (May & June), middle stage (July & August) and terminal stage
(September & October) respectively.

## Table 2. Medicines

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R₁</strong></td>
<td><strong>R₂</strong></td>
</tr>
<tr>
<td>Tabutrex (Di-n-butyl succinate)</td>
<td>Crag Fly Repellent (Butoxypolypropylene glycol)</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>DDT</td>
</tr>
<tr>
<td>DDT</td>
<td>Xylene</td>
</tr>
<tr>
<td>Versicol AR-60</td>
<td>Toximul-500</td>
</tr>
<tr>
<td>Noogen 10-OD</td>
<td>DDT</td>
</tr>
<tr>
<td></td>
<td>Xylene</td>
</tr>
<tr>
<td></td>
<td>Toximul-500</td>
</tr>
</tbody>
</table>

Notes: Versicol AR-60 and xylene were used as emulsifiers. Noogen 10-OD and Toximul-500 were used as solvents.

Use the solution after adding 20 parts of water to one part of the original solution.

2. Results of Investigations

A general view of the results is shown in table 1.

1) Group I (R₁)

Case No. 1 showed slight itchiness of the neck and tail before observation, but skin lesions were not recognized. At the initial stage, despite spraying of R₁ daily, the patient showed an increase in itchiness and increase in depilation of the neck area. In the middle stage, typical symptoms of "kasen" were observed; they continued to the terminal stage. The authors could not prevent the patient falling victim to "kasen". During the middle and terminal stages, spraying of R₁ was insufficient and sometimes the patient was pastured. Eosinophile of No. 1 showed gradual increase parallel with the symptoms becoming worse.

In case No. 2, R₁ was sprayed sufficiently. At the initial stage of the experiment, slight itchiness of the neck and tail, and short cut hair in the areas were recognized. No inclination of the symptoms to become worse was observed in the middle stage. Moreover, a decrease of itchiness and regrowth of the hair in the neck and tail were also seen. Eosinophiles trended to decrease in number in the terminal stage.

2) Group II (R₂) (Plate II)

Both animals of this group did not show any symptoms of "kasen" before the observation period. Spraying of R₂ had begun before the animals fell victim to "kasen". In the initial
stage, no symptoms were considered to indicate non-illness. In the middle stage of the observation, itchiness of the neck and tail appeared first followed by changes of the skin of the neck and tail which showed shortness of hair, crust, dandruff as typical symptoms of “kasen” in both cases. R2 could not give an efficient protection against “kasen”. Eosinophiles showed an increase in count in the middle stage.

When the symptoms became worse in the middle stage, the authors diagnosed that the disease could not be cured even though the spraying of R2 were continued. So, the spraying of R2 was stopped and as subsequent treatment R6 was applied. Consequently, the symptoms of the skin gradually turned for the better; there was decrease in itchiness of the skin, and the changes of the skin of the neck and tail became slight. In the terminal stage, the changes caused by the skin lesions recovered mostly. Eosinophiles decreased in count gradually.

3) Group III (Rd)

Rd was examined against 2 horses. In both cases, slight itchiness and falling off of hair of the tail were observed in the initial stage. In the middle and terminal stages of the observation, itchiness disappeared and also regrowth of hair was seen. Eosinophiles did not change in number in No. 5, while in No. 6 the cells showed an increase in number at the middle stage.

4) Group IV (Re) (Plate IV)

The two cases of this group were examined with spraying of Re during the whole course of the test. Symptoms of “kasen” were not recognized throughout all stages. The authors were able completely to prevent the patients falling victim to “kasen” in the summer season. Eosinophiles did not change in number in No. 7, but showed a gradual increase in No. 8.

As mentioned above, good efficiency was obtained in the cases where Rs was used; the cases did not fall into “kasen” at any time through the whole course of the observation. Rd was not an effective preventative against “kasen”, but symptoms of the cases were very slight in the initial stage, then almost recovered at the middle stage and the terminal stage. R6 was not effective against “kasen” to prevent falling victim to the sickness and also not effective for control of the disease which trended to become severe. One case of Rf showed similar reactions as those of Rd, but the other showed typical symptoms of “kasen”.

II. TESTS IN 1959

On the basis of the results obtained from the 1958 experiments, the authors reached a conclusion that Rs is the best preventive repellent against “kasen”. In the 1959 tests, Rs only, the same medicine as used before, was used against 5 horses. The results are as follows.

1. Materials and Methods

Materials and methods of experiment are shown in table 3.
TABLE 3. Experimental Methods and Results in 1959

<table>
<thead>
<tr>
<th>MEDICINE</th>
<th>CASE NO.</th>
<th>SPRAYING PERIOD</th>
<th>ITCHINESS</th>
<th>CHANGES OF LESIONS IN THE SKIN</th>
<th>CHANGES OF EOS. CELL COUNTS</th>
<th>RESULTS</th>
<th>REMARKS</th>
<th>TEST NO. IN 1958</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A  B  C</td>
<td>A  B  C</td>
<td>Decrease in B &amp; increase in C</td>
<td>Completely prevented</td>
<td>Sprayed sufficiently</td>
<td>7 (R₆)</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>±*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Increase in B &amp; decrease in C</td>
<td></td>
<td>&quot;</td>
<td>8 (R₆)</td>
</tr>
<tr>
<td>R₆</td>
<td>3</td>
<td>±*</td>
<td>-</td>
<td>-</td>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
<td>6 (R₆)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+** +**</td>
<td>Increase</td>
<td>&quot;</td>
<td>3 (R₄)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+** +**</td>
<td>Decrease</td>
<td>&quot;</td>
<td>4 (R₂)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>Increase</td>
<td>Typical symptoms of &quot;kasen&quot;</td>
<td>Not sprayed 2 (R₃)</td>
</tr>
</tbody>
</table>

Notes: A---Initial stage of the observation (May & June)
B---Middle stage of the observation (July & August)
C---Terminal stage of the observation (September & October)

* --- Itchiness was extremely slight.
** --- Changes of lesions in the skin were localized in the lower portion of the neck.
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1) Locality of experiment

The same district was chosen as in 1958 for the present experiment: Kotoni district near Sapporo.

2) Materials

Five horses were selected as the experimental animal; they had already been used in 1958 as case Nos. 3, 4, 6, 7 and 8. No. 2 of 1958 was employed as the control animal in 1959. Feeding and management of the 6 patients were the same as in the previous year.

3) Repellent and experimental methods

R₆ used in this experiment is the same medicament used in 1958. From middle of May to last of October, it was sprayed daily by the owners to the surface of the animals and their stables in the same way as before. Hematological and clinical observations were conducted 5 times at 30 days intervals. The whole observation period was divided into 3 stages as described above. Special management was not employed for the control animal and no medicines were applied.

2. Results of Investigations (Plates III and V)

The results obtained from the present experiment are shown in table 3. In 3 out of 5 cases, occurrence of "kasen" or falling victim to the disease was able to be prevented by use of R₆; the cases are Nos. 1, 2 and 3 in 1959. In the three cases, spraying of R₆ was conducted sufficiently during the whole course of the tests. Slight itchiness in the neck was observed in Nos. 1 and 3 early in the initial stage, but other clinical changes of the neck skin were not seen. Eosinophiles in No. 1 showed a decrease in number followed by increase, while in Nos. 2 and 3, they showed increase and then decrease.

In Nos. 4 and 5, spraying of R₆ was not so much as in the others. In No. 4, itchiness in the neck was observed at the initial and middle stages. With the beginning of the terminal stage, crust formation and depilation in the lower portion of the neck near the withers were recognized. Changes in skin were localized at only the neck area; they did not spread to the tail and other parts of the skin. Eosinophiles showed a gradual increase in number. In case No. 5, appearances of itchiness, depilation and crust formation were seen on the skin of the lower portion of the neck from the middle stage to the terminal stage. Changes of the tail and other skin areas were not recognized through the whole course of the observation; changes in the skin were localized to the neck portion as seen in No. 4. Eosinophiles showed a trend to gradual increase.

In the control case (Plate I) no repellent or insecticide was applied. Itchiness of the tail and neck portion and changes in the skin were observed at the middle stage, and typical symptoms of the disease were seen at the terminal stage of the course. Eosinophiles showed an increase in number compared with the experimental animals; moreover in the terminal stage a remarkable increase of the cells was observed.

As described above, from the tests in 1959 using R₆ only, in 3 cases the authors obtained good results: application of R₆ showed preventive effects against the horse falling victim to "kasen". On the other hand, in 2 cases, slight degree of changes of the skin in the lower portion of the neck was observed, while changes of the tail and the upper portion of the
neck were not seen. That is, spreading of the changes of the skin to the tail and other portions was stopped by the use of R8.

**DISCUSSION**

There are few reports concerning the application of repellents to domestic animals. HIXSON and MUMA, and HAYNES et al. reported that in cattle the examination of repellents consisting of pyrethrins and piperonyl butoxide against *Stomoxys calcitans* (L) obtained good results for preventing the insects provided spraying the repellents was done once or twice a week. TRAVIS et al. examined dimethyl phthalate, rutgers 612 and indalone against *Cluicoides*, and proved 4 hours minimum effectiveness. GRANETT et al. used a repellent or butoxypropylene glycol (M. W. 800) against *Cluicoides* and concluded that the medicine showed repellency of 5~6 hours duration. FINDLAY et al. applied indalone and formula 6:2:2 against *Cluicoides* and obtained more than 4 hours repellency. Further, APPLEWHITE and SMITH reported that dimethyl phthalate was the most effective repellent against *Cluicoides*. All the examinations on *Cluicoides* mentioned above were tested in the laboratory, not in field test. So, the present authors could not decide the degree of repellency for practical use.

The medicines used in the present experiments of both years are repellents and insecticide. R1 is comprised of 20% of Tabutrex and 10% of DDT in consistency. Tabutrex is recognized as a powerful repellent against *Stomoxys calcitans* (L), but it has no insecticidal power. DDT of course, has insecticidal power against *Stomoxys calcitans* (L) and also is an effectual deterrent to egg-laying by insects. Oleic acid has no potency against insects, but it does prolong the duration of effect to Tabutrex when used with it. Emulsifiers and solvents have no efficiency against insects. R2 is comprised of Crag fly repellent as main component and the molecular weight is 800. The repellent was found powerful against *Stomoxys calcitans* (L) and also has synergistic action with pyrethrins. R4 is common DDT solution. Main components of R4 were pyrethrins and piperonyl butoxide. The former is a well-known insecticide and the latter has synergistic effect in use with pyrethrins5; it is called a “synergist”. As synergists, isobutylundycylenamide 4 and 3, 4-methylene-dioxyphenoxy compounds4,5 are also applicable.

In the 1958 experiments, the authors obtained different results from using 4 kinds of repellents against “kasen”. Such results may be due to differences of repellency of the medicaments against insects and also to the methods of spraying of the medicines. Specially, in the examination in the open air, the results or effectiveness of repellents are mainly influenced by the spraying method. These phenomena were seen in the case of R1 (No. 1).
In the 1959 experiments, the most effective repellent, R₆, as indicated by the tests in 1958, was applied. The authors succeeded in protecting 3 patients from falling victim to “kasen”. Two cases showed slight degree of symptoms of the disease, because, it was considered, spraying of R₆ was insufficient. Characteristic signs of the disease were observed on the 2 cases. In them changes of the skin were confined in the lower portion of the neck without spread to the tail and other parts.

Comparison was made of the results obtained from the tests in 1958 and 1959. The animals which did not fall victim to the disease in 1958, also in 1959 did not display symptoms of the disease. To the contrary, the cases which showed symptoms of “kasen” in 1958, also showed the symptoms in 1959. The degree of the disease in 1959 was more slight than that in 1958. The authors consider that the fact may present an indication for treatment against “kasen”: continuous spraying of insecticides and repellents for several seasons may give good effects against the disease, and year by year, the gradual decrease in symptoms should result. Further, it is considered that if the spraying to patients with the medicaments is done more often and continuously, the occurrence of “kasen” will diminish.

It was already reported by the authors that eosinophiles show a gradual increase in number in parallel with the progress of the symptoms. In the present experiment, however, there were observed an increase of the cell counts irrespective of the symptoms of “kasen”, and a gradual decrease of the cell in number after the increase. These facts have led the conclusion that when one observes an increase of the cell count, the animal condition seems to trend to the worse, while by means of the use of the repellent, the patient is protected from the sting of insects, and an increase of the symptoms does not occur; eosinophiles also do not show increase in count and then the cell count shows a tendency to decrease gradually. Under the above mentioned conditions, it is thought that repellent control of the disease is possible, and it also seems that the medicine is an effective repellent against the disease from the hematological observation.

**Summary**

During the two summer seasons of 1958 and 1959, tests were conducted on the repellents prepared to protect horses from falling victim to “kasen”. The results obtained from the series of tests are summarized as follows:

1. In the experiment of 1958, repellents R₁, R₂, R₄ and R₆ were sprayed, during the period from middle of May to the last of October, on the body surfaces of 8 horses which had had “kasen” as a previous illness. It was found that R₁
was not protective against the disease but on the contrary showed effective for encouragement of the disease. \( \text{R}_2 \) could not be considered effective. \( \text{R}_4 \) was not effective in so far as prevention of the disease is concerned, but symptoms of the patients were limited slightly. \( \text{R}_6 \) showed the most effective action; the patients did not show any symptoms of “kasen” during the whole course of the test.

2. In the 1959 experiment, only \( \text{R}_6 \) was used for 5 patients. In 3 of the 5, the animals were prevented from falling victim to “kasen”; 3 patients did not show any symptoms through the whole course of the experiment. In the other cases, the authors could not protect the patients from falling victim to “kasen”; itchiness and changes of the skin were localized to the lower portion of the neck, and they did not progress to the tail and upper portion of the neck.

3. Through the observations of patients during 2 year test, the authors have reached the following conclusions; continuous application of \( \text{R}_6 \) by spraying yields good effect against the disease; the symptoms of “kasen” change toward the better; the degree of the symptoms observed in 1959 showed more slight than that of the same animals in 1958.

4. Finally, it is concluded from the results as a whole that spraying of \( \text{R}_6 \) daily during summer season is practically and preventively useful against “kasen”.

The authors wish to express their thanks to Prof. S. Yamagiwa, Dean of the Faculty of Veterinary Medicine and to Prof. J. Yamashita, Chief of the Department of Parasitology for their constant interest and valuable suggestions. The authors are greatly indebted to the Ministry of Education for its financial assistance. Likewise the assistance of the concerned authorities is highly appreciated.

The authors are also indebted to the Hokkai Sankyo Pharmaceutical Company who willingly supplied the medicaments and to Mr. Watanabe, a staff member of the research station of the company who offered many kind advices.

References

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EXPLANATION OF PLATES

PLATE I. Control case examined in 1959.
   Series A: Just before the observation (May 15, 1959).
   Series B: Middle stage of the observation (July 15, 1959).
   Series C: Terminal stage of the observation (September 15, 1959).
   Photographs in both series B and C manifest short cut hair on the neck area near
   the withers and lesions of the skin on the tail.

PLATE II. Examination of R2 (case No. 3 in 1958).
   Series A: Just before the observation (May 15, 1958).
   Series B: Initial stage of the observation (June 15, 1958).
   Both lesions of the hair and skin on the tail, and short cut hair in the neck near
   the withers are shown in the pictures of series B.

PLATE III. Examination of R6 (case No. 4 in 1959: this is the same patient, case
   No. 3 in 1958).
   Series A: Just before the observation (May 15, 1959).
   Series B: Middle stage of the observation (July 15, 1959).
   Series C: Terminal stage of the observation (September 15, 1959).
   In the pictures of series B and C, lesions of the hair on the neck area are seen,
   however no lesion of the skin is observed on the tail. (Comparison should be made
   with Plate II).

PLATE IV. Examination of R6 (case No. 8 in 1958).
   Series A: Just before the examination (May 15, 1958).
   Series B: Middle stage of the observation (July 26, 1958).
   Series C: Terminal stage of the observation (September 15, 1958).
   No symptom of "kasen" is shown in the pictures of each stage, the same as in
   case No. 7 in 1958.

PLATE V. Examination of R6 (case No. 2 in 1959: this is the same patient, case
   No. 8 in 1958).
   Series A: Just before the examination (May 15, 1959).
   Series B: Terminal stage of the observation (September 15, 1959).
   No symptom of "kasen" is shown in the pictures of series B, the same as in cases
   Nos. 1 and 3 in 1959. (Comparison should be made with Plate IV).