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THE INDIAN VETERINARY JOURNAL ¹⁷⁵⁸

(The Journal of the Indian Veterinary Association)

*A Monthly Record of
Veterinary Science*



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[THE JOURNAL OF THE INDIAN VETERINARY ASSOCIATION]

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A Monthly Record of Veterinary Science
Devoted to the cause of the
Veterinary Profession



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Editorial

ADMINISTRATION OF SCIENTIFIC DEPARTMENTS

The difference in the salary and status of the members of Administrative service and of those of Scientific services, has been a ripe cause for the non-development of several of the resources in our country. It is taken so much for granted that people are not able to realise that this *inferiority complex* imposed on Scientific men, pulls them down in their endeavour and enthusiasm. In more advanced countries scientific personnel receive equal if not better recognition at the hands of the Government. Their work is their exclusive privilege which none else could handle with equal knowledge and efficiency. They play a key role in the economic development of their country. In fact they hold the trump cards and can make or mar the future of their country. Their work is several times more arduous both mentally and physically. They have to work round the clock if scientific data are to be collected, collated, studied and applied in the field.

We ask then, is it in National interest to keep them ever suffering from this *inferiority complex*? It is hardly necessary for us to point out that this sense of *inferiority complex* will never permit a man to rise to his full heights in any walk of life. So instead of getting the best out of him, we merely allow him to drift on. Other countries have realised this and they treat their scientific personnel as a specially privileged class. Administrative Officers deserve all the attention they get and even more, if possible, but our contention is that the scientific personnel deserve in no way less. The sooner this disparity in administration is done away with, the better it would be for this country.

One way of erasing this *inferiority complex* is by reviving the old All-India Services for these professional men. We notice there has been some attempt in this direction, at any rate some talk, quite recently. We fervently appeal to the Government to bring about this much needed reform as early as possible. It would then be possible to effect inter-state transfers, whenever it is found necessary.

Another method of doing away with this *inferiority complex* is by not posting purely Administrative Officers as heads of Technical Departments and Technical institutions. This has had a very demoralising effect on professional men and wherever it was tried it was given up as an un-sound policy. This is neither good for the Administrative Officer nor for the technical department or college. This procedure has been condemned often by no less a person than the Prime Minister himself but yet the evil persists. The Indo-American Report has condemned it and yet the evil persists. We have ourselves pleaded in our own humble way, times without number in these pages and yet the evil persists. The Interim Indian Veterinary Council has condemned it and yet the evil persists. This anomaly saps out the efficiency and economy of the scientific departments. This evil is sought to be perpetrated under the plea that there are no suitable men within the Department to occupy such a senior post as the head of the Department. We have heard of this plea, shall we say, from time immemorial! The Britisher thought of this entire country as unsuitable for independence and yet she has earned a honoured place in the comity of nations. It is idle to trot out this argument any more. It has become a lame horse. As a doctrine it has come to stink in the nostrils of all honest men. The un-kindest cut of all is when this plea is agreed to or even adumbrated by men in our own profession and non-professional men are allowed to become the heads of professional Departments and institutions. This is the greatest harm that one can do to our profession. Posterity will not be slow to point the finger of scorn at such men.

General Articles

STUDIES ON LAPINISED-AVIANISED RINDERPEST VIRUS LABORATORY TRIALS

PART I

BY

M. S. MENON,

Indian Veterinary Research Institute, Mukteswar-Kumaon, U.P.

History:—After the evolution of Lapinised virus by Nakamura, *et al* (1938) for protecting all susceptible species of animals against rinderpest, the vaccine was tried in many countries in the World including India and was found quite suitable to be used on a large scale as a prophylactic measure against rinderpest. Certain breeds of cattle, however, have been found to be highly susceptible to rinderpest and cannot withstand even this mild strain of lapinised virus (Cheng and Fischman 1949). According to Nakamura and Kurdas (1941) 29 per cent of the Korean calves receiving the lapinised virus died of rinderpest, and even after several serial passages this strain did not show any evidence of progressive attenuation to Japanese native cattle and Korean cattle. Thus the lapinised virus was too pathogenic for these breeds of cattle to be used for the prophylactic vaccination. Secondly it was thought that the strain having got fixed in its character could not get attenuated any further for the safe use to these hypersusceptible breeds. Hence it was contemplated to attenuate the virus through a less closely related biological medium. From this view point serial passages of the virus through chicken embryos were considered worthy of interest and tried. After alternating intravenous passages in chicken embryos and rabbits and subsequent straight intravenous passages in chicken embryos, the lapinised rinderpest virus was found to be progressing in its propagating property and infectivity in chicken embryos, and a remarkable decrease in pathogenecity in rabbits and cattle was also observed. Thus Nakamura and Miyamoto (1953) were able to produce a lapinised-avianised strain and they designated as LA virus and LA strain respectively. Altogether the lapinised virus had undergone 330 chicken embryos and 41 conjunctive rabbit passages at various stages before this vaccine was considered suitable for their hypersusceptible stocks.

Immunisation of Korean cattle with LA virus:—In two preliminary experiments, (Reisinger, *et al* 1954) 4 Korean calves were inoculated with the original LA virus received from Tokyo. 5 ml. of a 1:500

suspension of embryo spleen produced only a slight thermal reaction and the animals were found to have acquired solid immunity on challenge by the bovine rinderpest virus. It was thought that LA virus would regain its original pathogenicity for calves on serial passage in rabbits but there was no indication of this as it was found quite non-pathogenic to 120 calves that had been inoculated in the laboratory with rabbit passage virus of high concentration and they survived the inoculation without any untoward reaction and revealed complete resistance to the challenge. More than 30,000 cattle were safely vaccinated in Korea during 1952 and 1953 with LA virus. No adverse reaction was observed in any of the vaccinated animals and more than 90% revealed the immune response by production of complement fixing and virus neutralising antibodies. Thus the Nakamura LA strain of rinderpest virus was found to be quite suitable, safe and effective and it conferred a solid immunity to Korean cattle and would be quite effective in immunising all species of hypersusceptible animals against rinderpest.

Receipt in India :—Lapinised-avianised strain was received from Tokyo in 4 ampoules of one gramme each through the courtesy of F.A.O. expert on 16th May 1955 at the Indian Veterinary Research Institute. On 17th May 1955 the viability and potency of this material was tested on two healthy hill bulls and four healthy rabbits. The healthy hill bulls were inoculated with 1 ml. subcutaneously of 1:100 suspension of the strain and the rabbits were inoculated *cc* 1 ml. each intravenously. These animals were kept under observation for a period of 21 days and later they were challenged with virulent bull virus in the case of hill bulls keeping two healthy controls and lapinised virus in the case of rabbits. It was interesting to note that neither the hill bulls nor the rabbits evinced any reaction as a result of challenge and stood the test firmly whereas the controls reacted and died of rinderpest. The work of propagating this strain in developing chick embryos could not be taken up for some time till the desired type of syringe, needles and other equipments were received. It took nearly two months to procure the above equipment. On 2nd July 1955 another ampoule of freeze dried LA virus was taken and its contents emptied in the pestle and mortar and a suspension of 1:100 in normal saline was made. Two healthy rabbits were inoculated intravenously *cc* 1 ml. each, on 2—7—1955, which were sacrificed for harvesting the material on 6—7—1955. The spleen and mesenteric glands from the infected rabbits were harvested and made into a suspension of 1:100 in 6% glucose and 0.05 ml. of this dilution was injected into the vein in the chorio-allantoic membrane of each 12 to 14 days incubated eggs. The inoculated eggs were incubated at 38°C for a period of 5 days. The live embryos after this period of incubation were dissected to harvest the

whole of the viscera and the material thus harvested was stored in the refrigerator in the ice chamber. For the further serial passage work, the spleen and liver of the infected embryos were only used. The enlargement and reddening of spleen and greenish changes of the liver are the characteristic changes in chicken embryos infected with the lapinised avianised virus. These changes were conspicuous in the material harvested from the chick embryos in the laboratory. One gramme of spleen and liver from the infected material was thoroughly triturated in 100 ml. of 6% glucose and 1000 units of penicillin and 5 mgms of streptomycin were incorporated in each ml. of inoculum and stored for an hour at 60°F to 70°F room temperature. The inoculum consisted of 0.05 ml. of the above suspension which was given always intravenously in 12 to 14 days developing chick embryos. Thus the serial passage of this strain was started. The viability and potency of this strain were checked periodically in rabbits as well as in cattle. At the initial stages when this seed material was titrated in healthy hill bulls, it was found that the virus gave a titre of 1:200 only, (Dhanda and Menon 1958). But on serial passage the titre of the virus began to progress gradually. The details of the titration tests indicating the number of passage and the increase in titre at various passage level are given in the following table.

TABLE I

No. of serial passage	Dilution	No. of hill bulls used	Results
5th wet	1:400	2	Immune
11th wet	1:800	2	Immune
17th wet	1:1600	2	Immune
33rd LA virus cryochem	1:200	2	Immune
dried	1:400	2	
	1:800	2	
11st wet	1:6000	2	Immune
	1:12000	2	Immune
	1:16000	2	Immune

The diluent used in titration was only normal saline solution. For each test the control hill bulls reacted severely and died of rinderpest.

Thus this strain of lapinised-avianised virus got stepped up gradually to a high concentration in the tissue of developing chick embryos. In order to find out the viability and potency of the freeze dried lapinised-avianised virus at passage level 33, which was lyophilised in December 1955 and stored in deep freeze cabinet at -20°C was reconstituted in normal saline after a period of 9 months storage in the dilution of 1:400 (original titre 1:800) and two hill bulls and two

rabbits were inoculated with 1 ml. of above suspension, intravenously in rabbits and subcutaneously in hill bulls. After a fortnight the hill bulls were challenged with virulent bovine rinderpest virus and it was found both of them were immune and the control reacted and died of rinderpest. This test had indicated that the virus in the freeze dried form remained viable and potent in the deep freeze cabinet at -20°C in sealed ampoules for at least 9 months the maximum period tested at that passage level.

Of the two infected rabbits one of them was sacrificed to harvest the material (spleen and lymph gland) for the propagation of a second line of passage in order to provide an alternative source of virus in case the first line was lost during the routine passage work. The virus of passage level 45 of the second line was titrated in hill bulls in dilution of 1:4000, 1:8000 and 1:16000 in the wet form and 1:400, 1:800 and 1:1600 in the freeze dried form using two hill bull for each dilution. All these bulls when later challenged proved solidly immune while the controls severely reacted and died of rinderpest. It showed the titre of the virus of the 2nd line of passage level 45 was at least 1:16000 in wet form and 1:1600 in the freeze dried form. The other rabbit after 21 days was challenged with lapinised virus and found to be solidly immune. From the first line at its 59th passage level lapinised-avianised virus was titrated in hill bulls in a higher dilution of 1:16000, 1:32000 and 1:64000, using 2 hill bulls for each dilution. After a fortnight when they were challenged with virulent strain of bovine rinderpest virus, all proved immune while the controls reacted and died. This experiment had revealed that this strain had got adapted to the embryonic spleen tissues in which it had attained a titre of 1:64000 in wet form.

The details of the test are given in table II.

TABLE II

Passage No.	Dilution	Date of vaccination	No. of hill bulls	Date of challenge	Result	
59	...	1 : 16000	1-5-56	2	16-5-56	Immune
"	...	1 : 32000	"	2	"	Immune
"	...	1 : 64000	"	2	"	Immune

Control animal typically reacted and died of rinderpest.
Keeping Quality and Stability test.

Keeping quality and stability of this virus at various stages and levels of exposure were studied with the freeze dried material at the

passage No. 33. This virus material at the above passage level was freeze dried in sealed ampoules on 24-12-55 and stored as follows:—

	Period.
(a) Deep freeze cabinet at -20°C	... 45 days
(b) On ice for	... 25 days
(c) At 80°F to 90°F in wooden box	... 15 days
(d) Deep freeze cabinet -20°C	... 20 days

As mentioned earlier this virus had an original titre of 1:800 in hill bulls. After the above various treatments 1 gramme of the freeze dried material from the ampoules was taken and a suspension of 1:100 was made in normal saline. Two healthy hill bulls were vaccinated at the rate of 1 ml. each subcutaneously of above suspension keeping 8 m.i.ds. in each vaccinating dose. These hill bulls were challenged after 15 days with bovine rinderpest virus keeping a control. It was interesting to note that these two vaccinated animals withstood the challenge and proved solidly immune whereas the control animal typically reacted and died of rinderpest. This experiment had shown that this virus is quite stable as compared with any other attenuated strains of rinderpest viruses.

In order to confirm the previous findings of titre in the viscera of the infected chick embryo in the course of further serial passages, titrations were carried out at the 137th passage level in hill bulls in the dilution of upto 1:64000 and it was found the virus maintained its titre, as well as the pathological changes in the viscera viz. the enlargement of the spleen upto 3 to 4 times of its normal size of spleen in the embryo, with redness and white necrotic spots in the enlarged spleen. These white necrotic spots in the enlarged spleen were not observed previously in the early stages of this virus passage. These changes in the spleen are not a constant feature, but it is invariably seen in very highly enlarged spleen of the infected embryo. Bacteriological examination had also given negative results for the presence of any pathogenic organisms.

At the 137th passage level the virus was titrated in hill bulls in the dilutions of 1:64000 using 2 hill bulls in the wet form, and 1:3200 and 1:6400 in the freeze dried form, using 2 healthy hill bulls for each dilution. These animals when later challenged with virulent bull virus proved immune to the challenge virus, while the controls reacted and died of rinderpest. On certain occasions when the supply of eggs fell short the passage material was stored in the ice chamber for about 8 to 10 days in order to resume the passage when eggs became available. In this case it was observed that the virus had to be passaged serially at least half a dozen times to get the original lesions in the infected embryos, as well as the virus titre. Hence it was found

necessary to passage the virus from egg to egg to maintain the exact titre. The titre of this strain at the 203rd passage level was again ascertained in hill bulls and confirmed to be at least 1:64000 in the wet form and 1:6400 in the freeze dried form in healthy hill bulls. Hence it was considered that the vaccine produced from this strain would be cheaper than any other rinderpest vaccine in the country. Earlier than this observation, experiments were conducted to determine the virus titre using the whole infected embryo. Embryonated eggs of 12 day old were used for this experiment. (Nakamura *et al* 1953). To start with 20 eggs were inoculated intravenously from the virus material harvested at the 109 passage level at the rate of 0.05 ml. in each egg and incubated for 5 days at 38°C. The survival rate after this period was only 50%. The embryos from the survived eggs were harvested, the beaks, wings and legs were removed, and emulsified in a Waring blender and 1 ml. of the suspension was titrated in this dilution of 1:1000 and 1:4000 in the wet form and 1:100 and 1:400 in the freeze dried form, using 2 healthy hill bulls for each dilution.

TABLE III

No. of passage	Dilution	No. of hill bulls	Date of Vaccination	Date of Challenge	Results
Wet 109	1:1000	269 } 2	30-1-57	2-3-57	Immune
"	1:4000	290 } 2	"	"	No. 290 Immune and 517 died of debility on 12th day
Freeze dried material at the above passage	1:100	217 } 2	"	"	Immune
	1:400	557 } 2	"	"	No. 557 Immune and 257 died before challenge due to debility

Control reacted and died of rinderpest.

These animals were challenged after two months (vide table III) and it was found that the animals which received the wet virus in the dilution of 1:1000 and the freeze dried 1:100 stood the challenge, whereas one animal in the dilution of 1:4000 and 1:400 died of debility thus giving inconclusive result. However this experiment had confirmed the findings of Nakamura *et al* (1953) that the tissue of the whole embryo also contains a high titre of the virus. The experiment was repeated at 171 passage level using the whole embryo after removing the legs, wings, and beak and grinding in the Waring blender for making the emulsion for titration.

1 ml. of the emulsion was titrated in the dilution of 1:16000 and inoculated into two healthy hill bulls 1 ml. subcutaneously in each of the hill bulls. After a fortnight these two animals were challenged with bovine rinderpest virus, and they were found to be immune while the controls died of rinderpest.

As the serial passage level advanced in the 12 to 14 days embryonated eggs, titrations were conducted periodically as mentioned earlier to ascertain whether the virus continued to grow in the tissue of the embryo or reached a stage at which it could not grow further. It has been found that the virus maintained its titre intact, excepting on one occasion when the supply of eggs fell short and the material had to be cryochem dried and stored in the refrigerator, as the available deep freeze cabinet was out of order. The stored back passaged wet virus material was also freeze dried in Edwards 3 P.S. and stored in the refrigerator. Titration was carried out from the above freeze dried material in the dilution of 1:800, and 1:1600 using two healthy hill bulls for each dilution. On challenge of these animals after a fortnight it was found that one animal of each group died of rinderpest while the others severely reacted and recovered. The breakdown of immunity had definitely indicated the great fall of titre or the entire absence of the virus. To make sure of the latter, titrations were further carried out in lesser dilutions than the previous ones *viz* 1:300 and 1:600 and using two healthy hill bulls for each dilution. It was very surprising to note that all the animals reacted very severely and died of rinderpest on challenge after a fortnight. This test had revealed that the freeze dried material had no virus, and either it was lost while at storage in the refrigerator or at the time of freeze drying. These findings have shown that virus material in the wet form stored in the refrigerator in the ice chamber for two to three weeks and then freeze dried in Edwards 3 P.S. lost its titre considerably or even lost the whole virus content. Therefore, it is essential that the wet material immediately after harvesting should be freeze dried for keeping up the virus content in a viable form. (Piercy and Witcom, 1947). In the process of freeze drying also, all virus preparations suffer a considerable loss of titre, (Daubney, 1954). "However, it is difficult to secure comparatively accurate figures while dilution intervals are commonly ten fold. By this ready and rough method it can be said that an apparant fall in titre of Log 1, representing a loss of 90% is the most that should be tolerated, that it is possible to reduce the loss at times to 50% and that 80% should be the normal figure." In face of this observation it is apparant that the wet virus material stored in the refrigerator for about two to three weeks and freeze dried in Edwards 3 P.S. did not contain the minimum protective dose.

TABLE IV

Freeze dried LA virus after 2 to 3 weeks storage in the refrigerator.

No. of passage	Dilution	Date of vaccination	No. of hill bulls	Date of challenge	Results
244 to 276 ...	1 : 800	7-4-59	296 } 2 436 }	21-4-59	296 died of rinderpest 436 reacted and recovered
	1 : 1600	"	253 } 2 459 }	"	253 died of rinderpest 459 reacted and recovered
	Controls	"	780 } 2 803 }	"	Both died of rinderpest
	1 : 300	8-5-59	667 } 2 622 }	3-6-59	Both died of rinderpest
	1 : 600	"	660 } 2 877 }	"	Both died of rinderpest
	Controls	"	51 } 2 55 }	"	Both died of rinderpest

At this stage it was thought that this strain had lost its viability due its not having been stored in the deep freeze cabinet. However series of tests were carried out with lyophilised back passaged material stored in the ice chamber of this refrigerator at the passage level of 221, 234, 236 and 243 in healthy rabbits as well as in healthy hill bulls. At first the lyophilised material at the 221st passage was made into a suspension of 1 : 200 in 6% glucose and centrifuged for 2 minutes at 2000 revolution per minute. This supernatant suspension was taken and 1000 units of penicillin and 5 mgm. of streptomycin per ml. were added and kept at the room temperature 60°F to 70°F for about one hour. The inoculum was injected intravenously into 12 to 14 days embryonated eggs @ 0.05 ml. amounts. The inoculated eggs were incubated at 38°F for period of 5 days. The survived embryos were harvested on the 5th day of incubation, the virus material viz. spleen, liver, lungs, intestines etc. was harvested and pooled. A suspension of 1 : 500 in normal saline was made and two healthy hill bulls @ 1 ml. each were inoculated subcutaneously. After 15 days, on challenge with virulent bovine virus these animals were found to be immune while the controls succumbed to the challenge virus. A similar test was carried out with the lyophilised material at the 243rd passage and found to be potent as the tested animals withstood the challenge and found to be immune. As regards the tests in rabbits for viability and potency two of them were inoculated intravenously 1 ml. of 1 : 500 suspension of the lyophilised back passage material as mentioned earlier, and kept them under observation for a fortnight. On challenge with virulent lapinised virus these rabbits were found to be immune whereas the control rabbits died of rinderpest.

The above tests have revealed the presence of the virus in the lyophilised material of 221st to 243rd passage level; and further passage work was carried out with this material from egg to egg till satisfactory. Pathological changes were observed in the viscera of the infected embryos. The enlargement of spleen 3 to 5 times bigger than the normal spleen with redness, deep greenish tinge in the liver, congestion in lungs, and intestines etc. are the indications of the high concentration of the virus. These changes became very conspicuous after 30 to 40 further serial passages in 12 to 14 days embryonated eggs through intravenous route. At 289th passage level titrations were carried out using the viscera of this infected embryos in the dilution of 1 : 8000, 1 : 16000 and 1 : 32000 in normal saline. This time four hill bulls were used for each dilution excepting the last one for which only three were used. These hill bulls were challenged with virulent bovine rinderpest virus after three weeks keeping two controls. All the animals under test withstood the challenge perfectly well and proved solidly immune whereas two controls reacted typically and died of rinderpest on the 9th and 10th day respectively after challenge. This test indicated the titre had come upto a satisfactory high level as it had come up at its early passage level. In order to confirm this observation further titrations were carried out in higher dilutions of 1 : 64000 and 1 : 128,000 using two healthy hill bulls for each dilution. It was interesting to note that the tested animals in the above dilutions stood the challenge dose after three weeks and proved solidly immune while one control reacted and died of rinderpest, the other severally reacted and recovered.

While this work was going on, a side line of work was also started in order to study the virus titre in the whole infected egg contents. Piercy and Witcomb (1957) have recorded experimenting with an avianised strain of bovine rinderpest virus that the Yolk Sac method of inoculation in 5 day old embryonating eggs and incubating them for 8 days and using the whole egg contents from live embryos for vaccine production would give a large quantity of viable and potent vaccine at a cheap cost. Since lapinised-avianised virus is a very mild strain. It was considered worth while to conduct experiments on the above lines. At 293 LA virus passage level in this laboratory, the virus material i.e. the viscera of the infected and survived embryos was harvested and made into a suspension of 1 : 300 in 6% glucose. Two dozen 5 day old embryonated hens eggs were inoculated at the rate of 0.5 ml. in each egg and they were incubated at 38°C for 8 days. On the 8th day only 10 eggs out of 24 (about 42%) were found to be alive. The contents of all these eggs were emptied in a Waring blender and emulsified. From this emulsion 1 ml. was titrated in the dilution of 1 : 2000 and 1 : 4000

TABLE V

No. of passage LA virus	Dilution	Date of vac- cination	No. of hill bulls	Date of chal- lenge	Result
289	1: 8000	26-5-59	2 } 20 } 44 } 41 } 1	17-6-59	All immune
"	1: 16000	"	44 } 31 } 30 } 4	"	All immune
"	1: 32000	"	48 } 47 } 36 } 3	"	All immune
Controls	"	"	28 } 49 } 2 58 }	"	Both died on 9th and 10th day due to rinderpest
305	1: 64000	8-8-59	74 } 88 } 2	28-8-59	Both immune
	1: 1,28,000	"	91 } 90 } 2	"	Both immune
Controls	"	"	208 } 209 } 2	"	208 died due to rinderpest. 209 reacted and recovered

using two hill bulls for each dilution. On challenge after three weeks with virulent bovine virus these animals were found to be immune, whereas the controls died of rinderpest. This result confirmed the findings of Piercy and Witcomb, (1957). However, to get conclusive data further experiments were carried out in a higher dilution. At 301 passage level one dozen 5 days incubated live embryos were inoculated in the same way as mentioned above and incubated for 8 days. This time 6 out of 12 embryos were alive. The whole egg contents were emulsified and 1 ml. of the emulsion was titrated in the dilution of 10^{-2} , 10^{-3} , and 10^{-4} using two hill bulls for each dilution. On challenge after 3 weeks it was found that the animals received the dilution in 10^{-2} stood the challenge, while one in 10^{-3} died of debility before challenge, and one in 10^{-4} died of rinderpest on the 10th day of challenge. This experiment was again repeated at 311 passage level using ten embryonated eggs of 5 days old. Out of 10 only 4 eggs were found to be alive after infection and 8 days incubation. The whole egg contents of the live embryos were emulsified and 1 ml. of the emulsion was titrated in normal saline in the dilution of 1: 32000, 1: 64000 and 1: 1,28,000 using two healthy hill bulls for each dilution. On challenge after three weeks all these animals were found to be immune excepting one in the dilution of 1: 64000 which died of debility.

These experiments have conclusively proved that the whole egg contents contain a heavy concentration of virus which could be used for vaccine production economically. The details of the above experiments are presented in the following table.

TABLE VI

Passage No.	Whole egg contents dilution	Date of vac- cination	No. of hill bulls	Date of chal- lenge	Results
293	1: 2000	24-6-59	52 } 2 62 }	16-7-59	Immune
	1: 4000	"	69 } 2 70 }	"	Immune
	Controls	"	68 } 2 589 }	"	Both died
301	10^{-2}	31-7-59	67 } 2 53 }	28-8-59	Immune
	10^{-3}	"	*89 } 2 82 }	"	*Died of debility Immune
	10^{-4}	"	*72 } 2 76 }	"	*Died of rinderpest Immune
	Controls	"	*209 } 2 208 }	"	*Died of rinderpest Severely reacted and recovered
311	1: 32000	23-9-59	251 } 2 253 }	16-10-59	Immune
	1: 64000	"	*231 } 2 226 }	"	*Died of debility Immune
	1: 1,28,000	"	155 } 2 156 }	"	Immune
	Controls	"	217 } 2 222 }	"	Both died of rinderpest

After ascertaining the minimum titre of lapinised-avianised virus in the whole infected egg contents by the Yolk Sac method of inoculation to be at least 1: 1,28,000 it was considered economical to produce this vaccine by that method. As this vaccine was found to be safe, effective and economical for protecting highly susceptible animals in hill areas, where goat tissue vaccine was found to be too strong, the production of this vaccine on a large scale was found to be a great necessity in the rinderpest eradication campaign in India. The production of lapinised-avianised vaccine by inoculation intravenously in 12 to 13 days embryonating eggs and incubating them at 38°C for 5 days and harvesting the viscera from the surviving embryos for vaccine production has been found to be very tedious and a time consuming process. Hence it was found necessary to produce this vaccine by Yolk Sac method.

The first batch of lapinised-avianised vaccine produced by the author in the Biological Products Division at the Indian Veterinary Research Institute, Izatnagar, by the Yolk Sac method gave a minimum titre of 1: 8000 in the freeze dried product (vide table VII.)

TABLE VII

Method of inoculation	Dilution	No. of hill bulls used	Result*
Yolk Sac	1 : 2000	HB Nos. 411 } 371 } 225 } 3	All immune
LA vaccine Batch No. 1	1 : 1000	HB Nos. 408 } 324 } 397 } 3	All immune
One gramme freeze dried material in petri dishes in Edward 3 P.S. was used for this experiment	1 : 8000	HB Nos. 326 } 234 } 389 } 3	All immune
Controls	...	HB Nos. 737 } 176 } 2	Both reacted severely HB No. 737 was sacrificed for the collection of material and 176 died of rinderpest

Lapinised-avianised strain passage No. 311 was used as seed material for the production of the above vaccine. At first this seed material was made into a suspension of 1 : 200 in 6% glucose and inoculated intravenously into 50 twelve days embryonating egg @ 0.05 ml. per egg. These eggs were incubated for a period of 3 days at 100°F. On the 3rd day the survived embryos (about 35 were found to be alive) were harvested and the viscera of the embryos were pooled and emulsified in a Waring blender. From 1 ml. of this emulsion a suspension of 1 : 300 was made in 6% glucose for inoculation through Yolk Sac. 125 embryonating eggs of 5 days old were obtained. These eggs were candled and marked at the air sac end by a small cross after ascertaining the position of the embryos. The marked portion of the air sac ends were cut out with a dental drill so as to allow the needle to pass. The material prepared as mentioned above was inoculated @ 0.5 ml. in the Yolk Sac of each embryonating egg. After inoculation the eggs were sealed at the cut end with cellophane tape and incubated for 8 days at 100°F. After this period the surviving eggs were collected (about 30%) and the whole egg contents were emulsified in Waring blenders for about 2 minutes. This emulsion was filled in 5 ml. ampoules @ 2 ml. in each ampoule for freeze drying. But in this particular case 5 ml. ampoules were short and only about 200 ampoules could be used. The material left over was freeze dried in petri dishes in Edward 3 P. S. freeze dryer, and ampouled @ 0.25 gramme in each ampoule. A total quantity of about 200 grammes of freeze dried lapinised-avianised vaccine was prepared by Yolk Sac method with a minimum titre of 1 : 8000 in healthy hill bulls. Since 40 m.i.ds. are incorporated in one vaccinating

dose 1 gramme of this freeze dried material gave 200 vaccinating doses. Thus from a total of 125 eggs used for vaccine production (only 35 eggs were found to be alive after 8 days incubation) 200 gramme of freeze dried vaccines, equivalent to 40,000 vaccinating doses were obtained and each egg gave about 320 doses of vaccine which is very cheap indeed.

Lapinised-avianised vaccine which was hitherto being prepared by intravenous method is being replaced by Yolk Sac method as the former is not practicable on a mass scale. By the yolk sac method the preparation of vaccine is simplified and large yields are obtained from each embryo. But in this method the dispensing of wet material @ 2 ml. in 5 ml. ampoules is necessary before freeze drying, in order to retain the high titre of the vaccine.

Discussion

Lapinised virus which was found suitable for protecting all susceptible animals to rinderpest, but it was found too strong for Japanese black cattle and Korean cattle. Therefore a milder vaccine than the lapinised vaccine had to be evolved to protect those animals. Lapinised virus was passaged through chicken embryos and rabbits alternately to start with, finally direct passage in 12 to 14 days embryonating eggs by intravenous route a milder vaccine was evolved which was named lapinised-avianised or L.A. virus. This vaccine was used on a large scale in Korea and about 30,000 animals were protected against rinderpest. No adverse reactions were observed in any vaccinated animals and more than 90% revealed immune response by production of complement fixing and virus neutralising antibodies.

This strain was received in India in 1955. On viability and potency test this strain was found to be viable and potent at a titre 1 : 200 to start with. But on serial passages in 12 to 14 days embryonating eggs it was found that the virus was propagating steadily and progressively. Titration tests carried out at various passage levels showed a minimum titre of 1 : 64000 in wet forms and 1 : 6400 in freeze dried form.

It was found to be essential to passage the virus straight into eggs without any storage, as storage of this material in the refrigerator reduces the titre of the virus in a very low level, and freeze drying of the same will deteriorate, the virus content still further. Therefore, it is necessary that the material after harvesting should be freeze dried immediately to maintain the high titre. The presence of the virus is indicated by the lesions in the infected embryos i.e., enlargement of the spleen, deep greenish tinge in the liver, congestion in the lungs and intestines. Sometime it was also observed that the enlarged spleen of the infected embryos bear white necrotic spots.

Titration carried out using the infected whole embryo revealed the presence of this virus in the tissues of the embryos in high concentration. The minimum virus titre in the viscera of the injected embryos so far estimated is upto 1:128000 in healthy hill bulls. The 311 passage material was used to propagate the virus through yolk sac method and found the whole egg contents gave a minimum titre of 1:128000 in hill bulls. Hence the preparation of lapinised-avianised vaccine was resorted to by yolk sac method. By this method the vaccine production is simplified and large yields are obtained from each embryo. In order to retain the high titre of the vaccine it is necessary to dispense the wet material in ampoules straight before freeze drying. The first batch of vaccine produced by yolk sac method by the author gave a minimum titre of 1:8000 in the freeze dried form. It is estimated that one egg would give more than 300 vaccinating doses keeping 40 m.i.ds. in each vaccinating dose. This vaccine has been found to be very effective for immunising highly susceptible hill cattle against rinderpest and very economical.

Summary

Lapinised-avianised virus gives a low titre at the start and it can be stepped up by serial passage in 12 to 14 days embryonating eggs by inoculation through intravenous route.

The lesions exhibited by the infected embryos are a constant feature and it shows the presence of the virus in the infected embryos. It is observed frequently that highly enlarged spleen in the infected embryos contains white necrotic spots in addition to the other pathological changes in the viscera.

Wet virus material stored in the refrigerator for 2 to 3 weeks loses its titre considerably while freeze drying and it is necessary to freeze dry the material soon after harvesting. It has been found that egg to egg passage maintains the virus titre without any deterioration and it had maintained the high titre even more than 300 serial passages in this laboratory. The minimum titre of the wet virus material (viscera of the infected chick embryo) is found to be 1:128,000. The virus titre estimated of the whole infected egg contents has reached up to a level of 1:128,000 and stressed the importance to use the whole egg contents for the vaccine production.

The methods of production of first batch of lapinised-avianised vaccine by Yolk Sac method is briefly described and the utility of this mild vaccine for protecting highly susceptible hill cattle is also discussed.

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AN OUTBREAK OF LEPTOSPIRAL INFECTION IN BOVINES IN MADRAS STATE

By

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The existence of this peculiar disease was brought to our notice by the District Veterinary Officer, Chingleput, during October, 1959. On investigation, it was found that the disease was confined to cattle of different age groups and was sudden in its onset in most cases. The symptoms noticed were a high rise of temperature (Up to 105° or 106° F.), lachrymation from both the eyes with partial or complete opacity of cornea, presence of frothy saliva in the oral commissures, grinding of the teeth, occasional rigors and marked dyspnoea with weakness of the hind limbs associated with general malaise. In a few cases stiffness of the limbs were also noticed, symptoms associated with pyrexia developing subsequently. In advanced cases the animal fell prostrate with subnormal temperature, and succumbed within twenty-four to forty-eight hours.

The disease was confined mostly to the Harijan colony of Irumaram and Sembalam villages of Chingleput district. The cattle population in both the villages is about 250. Rinderpest has been non-existent in this area since 1945, and the prophylactic vaccinations against Haemorrhagic Septicaemia, Black Quarter, and Anthrax are being carried out from time to time.

In all, there were 15 attacks and 15 deaths during the entire outbreak; eight ailing and dead animals were available for observation and study, and the last date of death was 28—2—1960. The outbreak subsided subsequently and no further attacks have been reported till now. All the 15 animals that died manifested the symptoms reported earlier. In none of the animals were Icteric symptoms noticed, and diarrhoea was inconsistent. One of the animals showed a few ulcers in the buccal mucosa while another showed oedema of the throat and jawl. Marked enlargement of the pre-scapular and pre-crural lymph glands was observed in one case. Necropsy was performed in eight cases, four

being done within about 2 to 3 hours of death. The gross lesions were alike in almost all cases. The lesions were as follows:—

Pharynx	...	Severely congested.
Larynx	...	Severely congested.
Trachea	...	Severely congested.

Gastro-Intestinal Tract:— Abomasum:— Intense congestion of the mucosa, associated with varying degrees of oedema and gelatinous degeneration. Small intestines were also involved, the contents being slightly sanguinous.

Liver	...	Intensely congested, and friable.
Spleen	...	Intensely congested, enlarged with edges rounded.
Heart	...	Myocardium presented a parboiled appearance, and endocardium showed linear haemorrhagic streaks.
Kidneys	...	Intensely congested, with a varied sized grayish white necrotic foci studded all over the kidney.
Bladder	...	Mucosa slightly thickened and oedematous and showed punctiform haemorrhages.
Supra Renals	...	Intensely congested.
Lymph Glands	...	Enlarged.
Brain	...	Congested.

Materials and methods

Smears from peripheral blood, impression smears from different organs such as liver, spleen, kidney, bladder, lungs and lymphatic glands of the affected animals, that were available for study, revealed neither micro-organisms, nor protozoan parasites, on Gram's and Leishman's staining.

Apart from differential counts from dried blood smears, no detailed haematological studies were made. Differential counts revealed a distinct leucopenia, particularly of the neutrophilic type in all the cases. This was associated with a relative increase of the mononuclear cells.

Biological test carried out by the Superintendent, Institute of Veterinary Preventive Medicine, Ranipet, in susceptible Buffalo calves and white calves with defibrinated blood, and saline suspensions of spleen and lymphatic glands from 4 cases proved negative for Rinderpest and Haemorrhagic septicaemia. This was confirmed by reports from the Principal, Madras Veterinary College, and the Director, Indian Veterinary Research Institute, Izatnagar.

Blood and organ suspensions from every one of the affected animals given parentally to experimental animals such as rabbits, guinea pigs, mice and pigeons failed to produce any recognizable bacterial or viral infections.

Suspensions of fresh brain stored at 4° C for 1½ months, examined bacteriologically, were found negative for *Listeria*.

Urine collected from two cases during post-mortem was highly coloured and faintly turbid, and revealed marked albuminuria. The urine sediment showed a high concentration of mono-nuclear cells and neutrophils, degenerate epithelial cells and occasional erythrocytes. Tests for glucose and blood were negative.

Chemical Examination of Materials

Stomach contents were collected and sent to the Principal, Madras Veterinary College, who reported traces of cyanogenic glucosides suggestive of plant poisoning.

Histopathological Examination of Materials

Materials such as pieces of spleen, liver, kidney, bladder, heart and lungs from 4 cases were sent preserved in 10% formal saline to the Principal, Madras Veterinary College, and to the Director, Indian Veterinary Research Institute, Izatnagar.

The Principal, Madras Veterinary College, reported that the changes in the kidney tubules and other tissues were suggestive of a blood borne toxin. The Director, Indian Veterinary Research Institute, Izatnagar, reported that the kidney sections revealed organisms indistinguishable from *Leptospira* in the tubules on Levaditis' method of staining.

In the last 2 cases, *i.e.*, the animals which died on 14—2—'60 and 27—2—1960, impression smears taken from bladder and kidney, when stained by Fontana's method revealed organisms simulating *Leptospira*. Specimens were sent from the animal that died on 14—2—1960 to the Director, Indian Veterinary Research Institute, Izatnagar, and the Principal, Madras Veterinary College. The Principal, Madras Veterinary College, reported that the specimens were negative for *Leptospira* and the Director, Indian Veterinary Research Institute, Izatnagar, reported positive for *Leptospira*. Materials such as urine, heart blood, emulsions of bladder, liver and kidney, were inoculated into Fletcher's and Verwoort Schuffner's and Noguchi's media and incubated at room temperature at 37°C, for 3 to 5 days.

They were examined daily by darkground and also smears made from the cultures when stained by Fontana's did not reveal any organisms. Sera from six bovines collected from Irumaran village were sent to Dr. Wolff, of Leptospiral Laboratory, Amsterdam, Holland, for Serological confirmation and his findings were as follows:—

Cattle Serum I titre with <i>L. sejroe</i> and <i>L. semaranga</i>	1 : 10
Cattle Serum II titre with <i>L. andaman A</i>	1 : 10
Cattle Serum II titre with <i>L. icterohaemorrhagiae</i> incomplete	1 : 30
Cattle Serum II titre with <i>L. semaranga</i>	1 : 80

Cattle Serum II titre with <i>L. hyos</i> (strain Mitis Johnson)	1 : 100
Cattle Serum III titre with <i>L. icterohaemorrhagiae</i> incomplete	1 : 10
Cattle Serum III titre with <i>L. andaman A</i>	1 : 30
Cattle Serum III titre with <i>L. semaranga</i>	1 : 30
Cattle Serum III titre with <i>L. javanica</i>	1 : 300
Cattle Serum III titre with <i>L. poi</i>	1 : 300
Cattle Serum IV, V., and IV negative reactions with all serotypes tested.	

Histopathological Examination

'We found indeed a number of organisms of which I am sure that these are *Leptospires*, although their form is not entirely conclusive.'

Discussion

The clinical symptoms of this outbreak may mislead one to think that it is Haemorrhagic septicaemia, since there were frothy saliva and in a few cases loose dung, with severe congestion of intestines. Blood smears and impression smears from spleen, liver, and biological and cultural tests proved negative for any known bacteria and cultural tests proved negative for any known Bacterial and Viral diseases. The ruminal contents, liver, kidney, spleen, were sent for chemical analysis and the results led us to think that it might be due to blood borne toxin.

The results of the Histopathological examination of the Kidney, gave us the first indication that the outbreak is one of *Leptospirosis*. Necropsy was performed in eight cases, and materials were collected and tested biologically, on guinea pigs, mice and rabbits and culturally in Fletchers and Verwoorts and Noguchi's media. In all the above methods, we were not successful to isolate *Leptospira* in pure culture. Further work could not be pursued as the disease has subsided by the end of February, 1960.

Leptospirosis amongst Cattle in Madras State has not been reported so far and this the first record of such a disease.

Summary

This disease first broke out among cattle and deaths in the village continued for 3 months. At first the symptoms were suggestive of Haemorrhagic septicaemia and it was eliminated by cultural and biological tests. The results furnished on the chemical examination of stomach contents led us to think that deaths were due to plant poisoning. Histopathological examination by the Director, Indian Veterinary Research Institute, revealed *Leptospira* in kidney sections. Attempts to isolate *Leptospira* in pure culture were however, unsuccessful, probably because of the time taken for conducting post-mortem and collection of materials for experimental study.

We are highly indebted to Dr. Wolff, Head of Leptospiral Research Laboratory, Amsterdam, Holland, for his serological typing and Histopathological Examination and The Director, Indian Veterinary Research Institute, Izatnagar, for histopathological examination of the materials sent.

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SOME OBSERVATIONS ON AN UNUSUAL DISEASE OF CATTLE POPULARLY CALLED "YELLOW DISEASE"

By

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Introduction

For sometime past we have been receiving reports of the presence of a disease condition in cattle, designated locally as "Yellow Disease" characterised by intense yellow discolouration of the visible mucous membrane and the inner covering of the external ears, from certain areas of West Bengal. At times there have been alarming reports of high morbidity, as well as mortality. Work was, therefore, undertaken some months ago to investigate into the etiology and remedial measures against this disease.

Laboratory examination of pathological specimens from a large number of animals, so far, carried out has revealed the presence of a Leptospira-like organism in the affected animals. The symptoms and other clinical features also suggest very strongly that the disease is of leptospiral origin.

Bovine leptospirosis was first diagnosed serologically by Savino and Rennella (1945-48). The authors reported infection with *L. hyos* from 20% of a group of cattle in Argentina. The Russian worker Nikolajev (1946) reported epizootics in cattle in the U.S.S.R., the species implicated was *L. grippotyphosa*. Simultaneously, Israeli workers isolated the causative leptospira which affected mainly adult cows, the species of the organism being similar to *L. grippotyphosa* (Bernkopf, 1946). Later on other species of Leptospira have been isolated from cattle. (Alston and Bloom, 1958).

Available literature reveals that in India the first report of presence of leptospirosis among bovines has been recorded from Uttar Pradesh (Adinarayanan, *et al*, 1960).

Methods and Materials

Up to date 5 animals, one cow and 4 calves, have been observed for clinical features. Detailed post-mortem examination has been carried out on two animals, both calves.

Pathological specimens from two calves have been histologically examined. The tissues from liver, kidney and spleen were stained by H & E method, Levaditi's method, and also by Fielding's improved techniques of silver impregnation (Fielding, 1941).

Biological examination was carried out using urine from 4 cases, all affected for over 8 to 10 days and inoculating young guinea-pigs (100-150 gms. weight) with 1 ml. of freshly collected urine intraperitoneally. Peritoneal fluid was drawn every 24 hours from the fourth day and examined with dark ground illumination.

Samples of urine, from about half a dozen animals suffering from this disease, were also put to laboratory test.

Routine tests were carried out to eliminate presence of parasitic infestation and other bacterial infection.

Results

Clinical Features :—In a typical case the disease starts with a high rise of temperature, often upto 106°F. The fever comes down on about the third day. Almost simultaneously with the rise of temperature there is marked anorexia and constipation. From about the fourth day there is indication of Jaundice characterised by yellow colouration of the visible mucous membrane. The intensity of jaundice varied from animal to animal. In the case where the animals died, the yellow colouration was of a spectacular nature. In the case which was observed to make a natural recovery, the jaundiced character was not so intense. Generally, the animals died within 10-15 days of onset of the disease.

As the disease progressed, in some of the cases, but not in all, the skin became dry, conchal cartilage of the ear became wrinkled and parchment like, and ulceration in patches appeared on the skin especially on the thigh near the axilla, on the flank and at the base of the tail. These ulcerated patches were irregular in shape and varying in size. Ultimately, the skin over these ulcerated areas sloughed off leaving a yellow coloured raw surface (Fig. 1). All affected cases exhibited marked emaciation and extreme dehydration of the skin. Sometimes, severe weakness led to prostration. The wrinkled skin of the ear became dry and cracks appeared in places and, frequently parts of the ear sloughed off (Fig. 2).

On post-mortem examination it was observed that the sub-cutaneous tissue was highly icterus and dehydrated. Cross section of skeletal muscles showed yellow rings around bundles of muscle fibres, the muscle tissue itself showing only slight yellow colouration. Liver was slightly enlarged and dark in colour. Gall-bladder was filled with thick gelatinous exudate having a dirty yellow colour. Kidney capsule was adherent and could not be peeled off easily; kidney-cortex was somewhat grayish white in colour. Spleen was markedly atrophied and in one case looked like a thin leaf.

Histopathological examination of liver tissue revealed disorganisation of the liver cell-cords and the detached cells were found distributed at random without conforming to the usual lobular pattern. Necrotic foci were observed around the central veins which contained plugs of cell debris sometimes completely filling up the lumen of the veins. Portal areas showed lymphocytic infiltration of moderate intensity (Fig. 3). In the kidney glomeruli seemed to be little affected. There was thickening of the capsular basement membrane, and the cells lining this membrane showed evidence of cloudy swelling. Epithelial cells of the convoluted tubules exhibited the most marked damage. There were varying degrees of degeneration leading up to necrosis. In many places the necrosed cells were found sloughed off into the lumen of the tubules (Fig. 4). There was no notable cellular infiltration in the interstitial tissue.

While H. & E. stained sections showed the histopathological changes, *Leptospira*-like organisms could be demonstrated only by Fielding's improved technique of silver impregnation. The present authors were, however, unable to maintain cellular architecture intact by the latter method. But the leptospires could be demonstrated fairly satisfactorily. The organisms were found to be of varying sizes, some present singly and some in clumps. These organisms were present both in the liver and the kidneys. Fig. 5 shows presence of *Leptospira*-like

organisms. Urine analysis revealed presence of albumin, acetone and bile.

Experiments in laboratory animals showed that out of the twelve guinea-pigs used for different specimens, one guinea-pig each from 3 of the groups died, the 'inoculation-death' period varying from 10 days to 24 days. The authors failed to observe any leptospires microscopically in the peritoneal fluid or peripheral blood of any of the experimentally infected guinea-pigs. None of them including the dead one, showed symptoms of Jaundice or any other clinical signs of illness except that one of the guinea-pigs which were infected with urine sample from an affected calf, which died after 24 days of inoculation exhibiting general dullness with distension of the abdominal cavity. Large quantity of light coloured watery exudate was found on post-mortem examination. The only other naked eye change on post-mortem was slight enlargement of the liver, which, incidentally, was also the case with the other two dead guinea-pigs.

Discussion

The results obtained so far would appear to show that leptospiral organisms are definitely implicated in, at least, some of the cases of the so-called "yellow disease." The preliminary findings reported above will not justify a sweeping claim that all such cases are of *Leptospiral* origin. A fuller investigation will have to be undertaken in order to ascertain whether or not it is a disease of multiple etiology. It may also be possible that certain other factors inter-play with the presence of *Leptospira* in all these outbreaks.

Summary

Results of preliminary investigations into outbreaks of a peculiar disease in cattle, known commonly, as "Yellow Disease", are reported. The findings so far obtained indicate very strongly the possibility that the disease is of *Leptospiral* origin.

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EXPLANATION OF TEXT FIGURES

- Fig. 1. Showing ulcerated areas in different parts of the body; the ulcers show signs of healing.
- Fig. 2. Showing sloughing off of parts of both the ears.
- Fig. 3. Liver showing disorganization of liver cell-cords and lymphocytic infiltration. Note severe degenerative changes in cells around the central vein.
- Fig. 4. Kidney showing (a) thickening of the basement membrane and epithelial crescent the cells of which show signs of degeneration and necrosis; (b) necrosis of the lining cells of the tubular epithelium.
- Fig. 5. Liver showing Leptospira-like organisms of varying sizes in between cells, placed singly as well as in clumps. The tissue was stained by Fielding's improved technique in which the configuration of cells could not be made.

AN ETIOLOGICAL STUDY OF "YELLOW DISEASE" IN CATTLE IN BIHAR

By

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Photosensitisation associated with jaundice in domesticated animals has been reported by several workers in India and abroad. Clare (1952) in his admirable monograph on photosensitisation listed a variety of disorders recorded in different parts of the world as being accompanied by photosensitisation, hepatic dysfunction and icterus. Theiler (1918) observed that this disease in sheep and goats (popularly known as Geeldikkop) occurred between November and April. The disease was non-contagious and the blood, liver, spleen etc. of the diseased animals were non-infective. He, however, showed by feeding experiment that the *Tribulus terrestris* was the source of toxic principle. Quin (1928) observed in Merino sheep intense generalised icterus, severe subcutaneous oedema and marked sloughing and disfigurement of face and ears when they were fed with brown wilted "dubbeltjie" (*Tribulus* plant) and exposed to direct sunlight. In other breeds such as Black head Persian, the lesions were confined to the unpigmented and exposed parts of the skin only. He (1929) also observed that the

"Yellow Disease"

By

M. S. DAS AND G. K. GHOSH



Fig. 1



Fig. 2



Fig. 3



Fig. 4

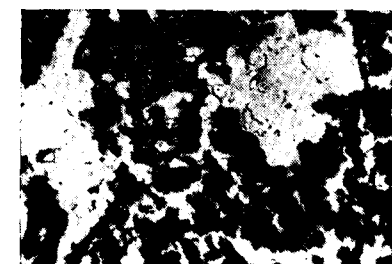


Fig. 5

"Yellow Disease" in Cattle in Bihar

By

LALA B. M. PRASAD *et al.*

Fig. 1

Case No. 4, showing extensive areas of exfoliation of skin of face, ear, neck and around eyes.



Fig. 2

Case No. 3, showing areas of necrosis and sloughing of skin around the vulva, hind quarter and abdomen.

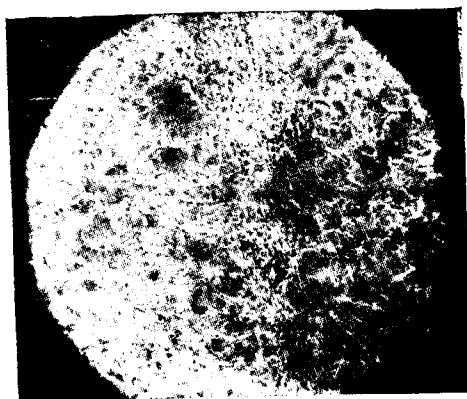


Fig. 3

Case No. 4 The liver cells in the centre have disappeared and their places taken by phagocytes.
H and E X 80.



Fig. 4

Case No. 3. Section of kidney showing an area of intertubular haemorrhage (upper margin). The blood vessel shows thickening of the media.
H and E X 80.

watery extract of the powdered Tribulus hay killed sheep in 75-90 minutes. Quin (1931) reported that haematoporphyrin could easily render a host of unpigmented animals (Guinea pigs, rabbits, sheep and goats) photosensitive if kept in the sun and that they could be protected by artificial pigmentation of skin with a number of dye-stuffs. He (1933) further succeeded in producing clinical jaundice and photosensitisation by ligation and severance of ductus choleductus in sheep when exposed to direct sun light.

Rimington and Quin (1934) while confirming the above findings reported on post-mortem enlargement of gall-bladder though the common bile duct was always patent.

Kehar (1938-39 and 1950-51) observed that goats when fed with 5-8 ozs. of Lantana plants (*Lantana-camara*) did not show any evidence of poisoning except loss of appetite whereas 12-16 ozs. of such leaves produced in cattle severe jaundice, dermatitis and exfoliation of skin on the muzzle, ears, thigh and tail. Icterus index was high in the serum of affected animals. Sahai (1938-39) investigated, in the Bhagalpur district of this State, into an obscure condition of cattle which was characterised by oedema of the face and dewlap and patchy necrosis of skin which was hard and leathery with loss of sensation. In a few cases, ulcers were noted on the lips, muzzle and the tongue. On post-mortem some showed a few liverflukes in liver and bile duct. The chemical analysis of viscera was reported negative and the examination of fodder and vegetations from the pasture failed to reveal Ergot. Blood of affected animals proved innocuous. Lal and Kalra (1960) failed to reproduce the lesion of dermatitis and jaundice in buffalo-calves, sheep and goats by oral administration of Lantana plant (*Lantana-camara*). In this State, several cases of generalised icterus associated with dermatitis have been reported in cattle during the last two years (Banerjee 1959, Pandey 1959 and 1960). Further it has been also observed that some of the castrated local Desi goats which are slaughtered at this station for the production of F.D.G.T.V. often show at autopsy intense yellow colouration of all the tissues and organs particularly during the months between December and June. Clinically however, these animals always appear healthy.

Material and Method

During the year 1959-60, 8 cases of severe jaundice and dermatitis were attended at Patna and its adjoining areas. A systematic investigation into seven such cases were carried out.

Case No. 1 :—This case was first attended on 4-8-1959. The animal affected was a cow, aged 4 years, of black colour with white patches in perineal and chest regions. The skin lesions were as a rule

confined to the unpigmented areas. Clinical symptoms manifested were those of dermatitis and icteric condition of all the visible mucous membranes. On the inner surface of the lips, there were numerous irregular, shallow yellowish ulcers on the way to cicatrisation. Ears were leathery to touch and patches of skin peeled off in shreds. The exposed surface was stained yellow. No rise in temperature was ever recorded. Urine was high coloured and bowel costive. The animal died while under treatment.

Case No. 2:—The animal affected was a brown cow aged 6 years and was attended to along with the case No. 1. Symptoms resembled those of the above except in that the degree of jaundice was not so intense. The animal recovered after treatment.

Case No. 3:—A grey heifer showing similar symptoms was examined on 24—10—1959. The skin showed wide areas of exfoliation. All visible mucous membranes, perineal region and teats were stained yellow. Both the ears were leathery. No rise in temperature was reported. Urine was high coloured and stool normal. The animal died.

Case No. 4:—This was examined on 28—10—1959. It was a cow of grey colour with symptoms of jaundice. Both the ears were leathery and skin scales were sloughing. No rise in temperature was reported. Urine was coffee-coloured. The animal died.

Case No. 5:—A grey coloured cow showing symptoms of intense icterus, leathery ears and sloughing of the skin. This was examined first on 4—11—1959. No rise in temperature was recorded. Urine was high coloured. The animal died while the treatment was in progress.

Case No. 6:—This was a cow aged 4 years, grey in colour showing symptoms of jaundice associated with dermatitis and was examined on 24—12—1959. The animal in this case showed symptoms of trembling of hind feet and disinclination to stand in direct sunlight. It very often sought shelter under shade. Both the ears were dry, tough and leathery. The skin lesions first appeared on the non-hairy parts of the body. No rise in temperature in course of investigation could be recorded. Urine was high coloured and stool normal in colour and consistency. The cow recovered eventually on treatment.

Case No. 7:—A cow aged 6 years with black and white patches on the skin was admitted as in-patient on 3—6—1960 at Civil Veterinary Hospital, Bankipore with symptoms of jaundice and dermatitis, the latter confined to the unpigmented parts of the body. The animal used to shiver and tremble under direct sunlight and had developed a tendency to seek shade. During the first three days of illness, the animal had rise in temperature (103°F) and had a tendency to lick the hooves. On enquiry from the owner it was learnt that this cow used

to feed capriciously on Lantana plant. The cow recovered on treatment.

Case No. 8:—A she-buffaloe aged about 6 years was admitted as an in-patient on 26-6-'60 at Civil Veterinary Hospital, Bankipore. The clinical symptoms manifested were intense yellowish discolouration of all the visible mucous membranes and perineal region. No skin lesion was present. Urine was high coloured and stool normal. The animal died on 27-6-'60 while under treatment.

Treatment

The general line of treatment adopted in all the cases were as follows:—

- (i) Dextrose saline 5% — 100 c.c. daily intravenously.
- (ii) Vitamin C— 10 grams daily, orally.

In case Nos. 1 & 2, Babesan 2 c.c. subcutaneously daily for 2 days were also given along with the above on empirical grounds.

Result of treatment:—Case Nos. 2, 6 & 7 recovered while the others died.

Post-Mortem Lesions

The common post-mortem lesions noticed were intense yellow discolouration of the subcutaneous tissue, fat, muscles, liver, kidney and other internal organs. In most cases the blood was dark red in colour. In all the cases the gall bladder was found distended and reached an average size of about 4" in diameter. They contained very thick dark green ropy bile mixed with mucous. However, in case No. 5, the gall bladder was distended to the size of a foot ball approximately of about 10" in diameter and contained thin yellow and viscid bile. In this case the neck of the gall bladder was found thickened. The common bile duct was found to be patent in all cases and in no case any obstruction could be detected either in the biliary passage or at the neck of the gall bladder. The contained bile invariably leaked out along the biliary passage on applying pressure though there was no free communication. In case No. 8, several amphistomes were found lodged in the bile duct.

As this was an obscure disease, the problem was attacked from all possible angles to incriminate bacteria, viruses, rickettsiae, protozoa and toxic plants as the causal factor. In course of clinical investigation, a detailed study of blood, urine, blood serum and stool were carried out on all the cases. For histological preparations, specimens from liver and kidneys were fixed in 10% formol-saline and the sections from paraffin embedded material were cut at 6 microns and stained with Ehrlich's Acid Haematoxylin and Eosin, van Gieson and Gomori's method for reticulum.

Histopathology

In general the liver cells looked normal. The bile ducts at the large portal tract showed slight hyperplasia. There was a slight increase in the number of mononuclears along the biliary radicles and a few polymorphs were also present. No necrotic changes were seen. However, in case No. 4 the blood sinuses were slightly congested and the Kupffer cells were prominent and at places showed mitotic figures. There was neither increase in the connective tissue fibres nor was there any frank area of necrosis. But at places liver cells had disappeared and their places taken by lymphocytes, large mononuclears indicative of regenerative process. In general liver cells in all the cases contained lakes of bile pigments.

In kidney, glomerulus and tubular epithelium were normal though the tubules were slightly dilated and contained hyaline and epithelial casts and leucocytes, at places almost occupying the tubular lumen. Several areas of focal haemorrhage in the intertubular connective tissue could be seen. Blood vessels showed considerable thickening of the tunica media.

Clinical Investigation

(i) *Blood*:—In general the haematological study showed pronounced anaemia and achromia. The total red cell count indicated a considerable fall in the number averaging from 3 to 3.3 millions per cubic millimetre. The number of leucocytes was also reduced to an average of 3 to 4 thousand per cm.

There was reversal of Neutrophil-Lymphocyte ratio. The haemoglobin content of blood ranged from 40% to 70%. The blood serum was invariably intense yellow in colour. On direct Vanden Bergh test, case nos. 3 and 4 gave strong direct positive reaction. Case No. 7 gave a weak positive indirect reaction. The microscopical examination of blood smears stained with Leishman, Giemsa and Macchiavellos' stain showed in all cases several azurophilic intracellular bodies about 0.5 to 1.0 micron in diameter invariably situated in the cytoplasm of the monocytes and large lymphocytes. They were mostly spherical or bipolar in outline and occasionally irregular in shape. In a few cases, the lymphocytes had ruptured giving suggestion of release of these intracellular bodies into circulation. In no case, such bodies were seen in the red cells.

(ii) *Faeces*:—The stool in general was dry and hard. Examination of faecal samples did not reveal the presence of parasitic infestation though in two cases (Nos. 2 & 5) very rare ova of *Haemonchus* and *Trichuris* could be detected.

(iii) *Urine*:—Urine was invariably brownish and turbid. In a few cases it was coffee-coloured. Albumen was present in heavy amounts in each case as also were the bile pigments. It was negative for sugar and red-cells. It gave strong positive reaction for porphobilinogen in case Nos. 3, 4, 6 and 7 and weak reaction in others. Ketone bodies were present in case Nos. 2, 3, 4 and 6. The results are given in Table No. 1.

Bacteriological Investigation

In all cases, the materials from blood, spleen, lymph-glands, liver, bone marrow, necrosed skin, urine and bile were seeded on to the routine culture media such as plain agar, blood agar, Mac Conkey agar plates, nutrient broth etc. and were incubated aerobically, anaerobically and in the presence of carbon-dioxide. In no case any pathogenic organism could be isolated.

The materials from heart blood, spleen and bone marrow were further inoculated into 6 days old embryonated eggs. The pooled samples were triturated in sterile distilled water and penicillin was added (4,000 units per ml.) This constituted the inoculum which was inoculated into the allantoic and amnion cavities and yolk sac. Materials were harvested at 24 hours, 36 hours and 48 hours. In no case, was there any macroscopic evidence of virus infection. Further, an ethereal extract of the harvested materials was prepared and used as antigen for the agglutination and precipitation test using the early and convalescent sera of naturally occurring cases. This was also used for routine H.A. and H.I. tests. In all these tests, results were uniformly negative. The presence of *Leptospira* was particularly looked for in urine and internal organs but with negative result.

The possibility that this condition might be associated with rickettsial infection was also investigated. For this purpose pooled samples of heart blood, liver, spleen, and lymph glands from each case were triturated in sterile distilled water and injected subcutaneously in the dose of one c.c. to a pair of calves, rabbits and guineapigs. None of them showed any reaction upto one month the period so far observed. There was no sign of scrotal reaction in male guineapigs which could be regarded as suggestive of rickettsial infection.

Ticks (*Boophilus* and *Hyalomma* spp) collected from the bodies of case Nos. 3 and 4 were fed on to two rabbits for four consecutive days with a view to incriminate the role of these vectors in the transmission and spread of the disease. These rabbits, however did not show any evidence of infection and remained normal throughout.

Materials such as spleen, lymph gland and yolk tissue of chick embryo infected with case No. 4 which were bacteriologically sterile were fed to two calves. Further the tissue suspension of these materials was also inoculated intranasally in two calves. No untoward reaction was noticed in any of these animals upto one month.

Protozoan Investigation

Special attention was also paid for the possibility of babesiosis, theileriasis, anaplasmosis and other diseases. Microscopical examination of smears taken from the heart blood, lymph gland and spleen which were stained with Leishman and Giemsa failed to reveal any protozoa.

Toxicological Investigation

Young flowering plants of *Lantana* spp were fed to four young grey calves for ten days from 6-1-'60 to 16-1-'60 @ 16 ozs. daily. This was mixed with green berseem. During and after the feeding period they were continuously exposed to direct sunlight daily. In no case, any sign of jaundice and dermatitis was noticed upto the period of two months.

Discussion

Cameron and Oakley (1932), Cameron, *et. al.* (1957) have shown that sudden occlusion of the common bile duct results in extensive necrosis of hepatic cells and progressive bile duct hyperplasia in liver. Recovery from liver damage caused by biliary obstruction has further been shown to occur after spontaneous restoration of the obstructed common bile duct (Trams & Symeonidis, 1957, Cameron & Prasad, 1960). Under such conditions the amount of serum bilirubin and alkaline phosphatase increases which shows close correlation with the extent of liver damage and the degree of jaundice (Cameron and Muzaffar Hasan 1958, Prasad 1959). In our cases the macroscopic appearance of liver always remain normal though jaundice persisted until death. In no case our sections showed any morphological alteration in liver suggestive of biliary obstruction. In case No. 4 however, the cellular pattern found in liver section was indicative of recovery from mild cholangitis. Therefore, generalised icterus in our cases could not be attributed as being due to obstructive jaundice nor could the normal liver be thought of as a result of spontaneous recovery. Unfortunately the estimation of serum alkaline phosphatase could not be attempted in our investigation which would have thrown further light as to the extent of disturbance of liver function. Nevertheless we strongly feel that the photosensitising agent which may even

be a normal constituent of bile increases in circulation following impediment in the bile outflow and dysfunction of liver as observed by Quin (1933) in sheep. In one of our cases (case No. 8) and also in that reported by Sahai (1938) the apparent cause of obstruction of bile outflow appeared to be due to the lodgement of parasites in biliary passage. Whatever be the cause of jaundice, it appears that as the bile fails to be discharged in small intestine, the concentration of the photosensitising agent increases in the system thus rendering the animal susceptible to the actinic rays of the sun. In cases, where there was no obvious biliary obstruction the bile was considerably concentrated, thick and mucoid in consistency, which may not have found free exit to the intestine under the existing bile pressure, thus probably accounting for the pathogenesis of the disease.

Our transmission experiment with the materials from the sick or dead animals failed to re-produce the condition in natural host or the laboratory animal. Though this work needs further to be extended in a variety of hosts by different routes, the result so far obtained shows that the materials were not infective and perhaps the disease is one of the metabolic disorder and liver dysfunction. Negative response in guinea-pigs and failure of serological reactions do not lend support for any contagion particularly rickettsiae. Perhaps the intracellular bodies in the leucocytes were the expressions of some toxic syndrome. This however, needs to be further confirmed by several blind passages in eggs and guineapigs.

From the stand point of ecology of the condition, as studied in 8 such cases and also reported by Sahai (1938-39), Banerjee (1959), Pandey (1959) and other workers, it appears that there is a seasonal periodicity of this malady, majority of cases appearing between October and June. This is also supported by the observation of workers of South Africa in a study of a similar disease in sheep (Geeldikkop). There had been heavy interrupted rain (see Appendix I) followed by hot weather which was particularly noticed as and when the cases appeared. It is quite logical to apprehend that during this weather all the vegetations specially the toxic ones (*Lantana* and others) were at the vulnerable stage. The ingestion of such plants may have yielded in the system of animals photosensitising substance, which under the influence of climatic factors produced dermatitis and jaundice. The susceptibility of unpigmented part of the skin to severe dermatitis was very striking. The case No. 7 which was a cow with mixed patches of black and white pigments showed dermatitis only in the unpigmented and exposed part of the skin. Also the buffalo-cow (case No. 8) which was darkly pigmented throughout showed no skin lesion. This shows that only the unpigmented skin is unduly sensitised by the photosensitising agent

or perhaps the black pigment exerts the protective influence against the actinic rays of the sun.

In our feeding experiment with *Lantana* plants we failed to reproduce the disease. It might well be that as there was no rain earlier to feeding, the leaves and flowers of the test plant was non-toxic. The failure of Lal and Kalra (1960) in reproducing the condition by feeding plants of *Lantana-camara* to buffalo-calves may also be explained on similar lines, though the weather conditions when their animals were fed have not been reported. In addition it might be assumed that the pigmented hosts i.e., the buffalo-calves which they used in their experiment may have remained refractory to the action of the photosensitising agent. This however, needs further confirmation by feeding a variety of hosts under suitable climatic conditions. The plant of *Lantana* spp. is found in abundance in this State and goats relish to feed on them. Despite this, they do not show any evidence of poisoning except jaundice. This may perhaps be explained as being due to biological variation and increased species tolerance.

The fluorescent pigment-porphrin, which is closely related to phylloerythrin and is a disintegration product of chlorophyll under the influence of bacterial fermentation in the forestomach of the ruminants, has been found to cause even with a minute amount (40 mgm) typical photosensitisation in sheep (Quin, Rimington and Roets 1935). They also observed that when the ruminal microflora especially *B. coli* was incubated with fresh green plant juice or in sterile broth to which a small amount of commercial chlorophyll has been added, phylloerythrin could be detected. In this context therefore the strong positive reaction for porphobilinogen in the urine of 4 out of 8 cases is rather interesting. So it is possible that the photosensitising pigment phylloerythrin of plant origin derived from chlorophyll is also responsible in a similar way in the pathogenesis of the "Yellow Disease" in cattle.

Future investigations aimed at the sorting of interaction between metabolic processes and the toxic principles of plants, specially phylloerythrin, might give the answer to the problem.

Summary

1. A hitherto unknown disease of cattle "Yellow Disease" has been fully described and its literature reviewed.

2. The disease is characterised by absence of fever and intense generalised icterus associated with dermatitis and sloughing of the unpigmented and exposed part of the skin.

3. The role of climatic factors in the causation of the disease has been stressed. The majority of cases appear between October to June.

4. The pathogenesis of the disease has been discussed. Though the precise etiology of this condition still remains to be elucidated, this does not seem to be concerned primarily either with biliary obstruction or any contagious vivum. Some toxic principle of plant origin has been suggested to be the etiological factor responsible for this condition.

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APPENDIX I

Rainfall chart.

Month-August, 1959				Month-October, 1959			
Date		Rainfall in mm.		Date		Rainfall in m.m.	
1-8-60	...	...	nil.	1-10-59	...	...	7.2
2-8-60	...	...	22.2	2-10-59	...	...	85.2
3-8-60	...	...	8.0	3-10-59	...	...	38.4
4-8-60	...	...	9.0	4-10-59	...	...	14.2
5-8-60	...	...	nil.	5-10-59	...	...	5
6-8-60	...	...	0.6	6-10-59	...	...	4
7-8-60	...	...	nil.	7-10-59	...	...	0
8-8-60	...	...	11.6	8-10-59	...	...	nil.
9-8-60	...	...	22.4	9-10-59	...	...	nil.
10-8-60	...	...	14	10-10-59	...	...	nil.
11-8-60	...	...	nil.	11-10-59	...	...	nil.
12-8-60	...	...	1.0	12-10-59	...	...	9
13-8-60	...	...	8.4	13-10-59	...	...	nil.
14-8-60	...	...	18.6	14-10-59	...	...	nil.
15-8-60	...	...	6.6	15-10-59	...	...	nil.
16-8-60	...	...	10.6	16-10-59	...	...	nil.
17-8-60	...	...	6.2	17-10-59	...	...	nil.
18-8-60	...	...	169.8	18-10-59	...	...	nil.
19-8-60	...	...	57	19-10-59	...	...	nil.
20-8-60	...	...	8	20-10-59	...	...	nil.
21-8-60	...	...	nil.	21-10-59	...	...	Trace.
				22-10-59	...	...	9.4
				23-10-59	...	...	nil.
				24-10-59	...	...	nil.
				25-10-59	...	...	nil.
				26-10-59	...	...	6.0
				27-10-59	...	...	0.2
				28-10-59	...	...	nil.
				29-10-59	...	...	nil.
				30-10-59	...	...	7.5
				31-10-59	...	...	nil.

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Clinical Article

TREATMENT OF *BABESIA GIBSONI* INFECTION IN DOGS WITH 'SPIROTRYPAN' HOECHST

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Introduction

Patton in 1910 described *B. gibsoni* infection in the hounds of the Madras hunt and the jackals in that region and later it has been found to be a widespread parasite in dogs in certain parts of India and Ceylon. Apparently there are no authentic records of the incidence of this parasite outside India and Ceylon, though Neitz and Steyn (1947) and Neitz (1956) consider that the *Babesia* described as occurring in the wolf, *Canis lupus*, in Turkestan, the Indian wild dog, *Cyon dukhnesis* and the fox, *Vulpes dorsalis* in French Sudan are synonymous with *B. gibsoni*. Further, the administration report of the Imperial Veterinary Research Institute, Mukteswar for the year 1935-36 states that a medical officer working in China had sent blood smears from a dog showing parasites indistinguishable from those of *B. gibsoni*.

Symons and Patton (1912) reported that a single injection of Salvarsan, given intra-muscularly, cured completely 25 hounds out of a total of 40. However, there was a fair amount of local oedema and necrosis. Five of the dogs so treated died and some of them had complications. Later Symons (1928) used neo-salvarsan, or novarsenobillon intravenously in preference to salvarsan because of the necrosis produced by the latter drug. He reported that Tryparsamide was not as effective as the other arsenicals. Stirling (1927) on the other hand reported that repeated injections of Tryparsamide gave highly satisfactory results. He recommended 5 injections and in some cases up to 8, at 5 day intervals. The dose adopted was approximately 1 gm./10 kg. body weight of the drug per injection given in saline as a 20 per cent solution. Rao (1927) tried various drugs and found that mercurochrome-Icthaeron, antimony tartarate, and sobita in oil had no appreciable action while novarsenobillon, neosalvarsan and Tryparsamide produced satisfactory results both therapeutically and prophylactically. Novarsenobillon and neosalvarsan were used in doses of 0.4 gm.

per adult dog, while Tryparsamide was used in doses of 2 gm. in 20 ml. per dog. Trials carried out at the Imperial Veterinary Research Institute in Mukieswar in 1932-1933 with the proprietary drug 'S.U.P. 56' indicated that it had no action, while trials made in 1933-34 with novarsenobillon, neosalvarsan, Antimosan, and quinine hydrobromide gave varying results. Tryparsamide was used in 1934-35 and trypaflavin and Akiron R (Acaprin) in 1935-36. None of these drugs could produce sterilisation of the circulating blood.

Shetty (1931) tried Sulfarsenol in two cases with success. Kapur (1943) in a comparative study of the actions of novarsenobillon, sulfarsenol and Tryparsamide involving 27 dogs found that novarsenobillon was the best drug when given at the rate of 0.01 gm. per lb. body weight. Tryparsamide gave the poorest results. With each of these drugs a course of four injections was given.

Babesia gibsoni infections in dogs in Ceylon are extremely common. The Veterinary Research Laboratory, Peradeniya reports finding 40, 52, 106, 57, and 63 positive blood smears for *Babesia gibsoni*, as against 2, 1, 0, 1, 2 cases of *B. canis* infections in the years 1948, 1949, 1950, 1951 and 1952 respectively. (Administration Reports of the Director of Agriculture for 1948-52) and the records at the Govt. Veterinary Hospital, Peradeniya for 1959 and 60 show that 112 and 124 positive cases of *B. gibsoni* infections were treated at this institute as against 6 and 5 positive cases of *B. canis* infections respectively.

Tryparsamide has been employed widely in Ceylon for the treatment of *B. gibsoni* infections. However, results have not been very satisfactory even when up to seven injections were employed. In such cases neoarsphenamine B.P.C. (novarsenobillon; N.A.B. M. & B.), has been employed. This drug has been found to be more effective but rather toxic. Under these conditions a more effective and a safer drug has been sought for during the last several years. Pillai (1953) used paludrine lactate intravenously in 25 cases and recorded good results. Five deaths occurred, and the others were cured. Seneviratna (1953) reported treating 21 cases of *B. gibsoni* infections with 0.05-0.2 gm. Paludrine hydrochloride orally, daily, according to body weight for 10 days. Of these cases, 17 recovered and one died. There was no appreciable improvement in the other three cases. Subsequently many cases have been treated with a combination of Tryparsamide given at 1 gm. per 10 kg. body wt. intravenously, every 4th day combined with the above doses of paludrine hydrochloride. Usually 3-4 injections of Tryparsamide had to be given. This regimen of treatment has been found to give satisfactory results though clinical evaluation of the combined actions of the two drugs has not been undertaken. However it has been found that paludrine hydrochloride sometimes causes nausea and

jaundice, if this is not judiciously used. Therefore during the past few years more effective and safer drugs were always sought for, to treat *B. gibsoni* infection. Various antimalarials such as Nivaquin, pyrimethamine, ('Daraprim' B.W. & Co.) and even Tetracyclines were tried in a limited number of cases, without much effect. Various arsenicals like sodium caparsolate and 'Spirotrypan' Hoechst were then used. Of these drugs 'Spirotrypan' 10 per cent solution given intravenously has given the best results, and appears to be superior to all other drugs employed in the treatment of *B. gibsoni* infection.

'Spirotrypan' Hoechst is a complex organic arsenic compound having an action similar to Salvarsan and to neoarsphenamine B.P.C. with the chemical formula: Sodium 2-di (β - ∞ - dihydroxypropyl) aminophenol - (4 - arseno - 5) - β - (benoxazoyl - (2) - mercapto) propionate.

The purpose of this communication is therefore to record the results of the therapeutic trials made with this drug.

Certain criteria were used to assess cure. Before these are mentioned it is necessary to enumerate the main clinical signs of *B. gibsoni* infection. The main clinical signs are: (I) A fluctuating temperature which may reach upto 106°, remaining high for several days and dropping to normal or sub-normal even though the animal is acutely ill. (II) Progressive anaemia. (III) A marked loss of appetite even when the temperature has dropped to normal. (IV) Enlargement of the liver and spleen. Usually the spleen is much more enlarged than in any other disease of the dog. (V) Microscopic examination reveals the presence of parasites in the blood. The number of parasites in the circulating blood varies considerably from day to day (Symons & Patton, 1912) and in certain instances even when other clinical evidence of *B. gibsoni* infection is present, parasites may not be found in the peripheral blood.

Therefore the criteria for cure were based on all the factors enumerated above.

In preliminary trials it was established that the maximum dose of Spirotrypan which was uniformly well tolerated by all ages and breeds of dogs under varying conditions did not normally exceed 1 ml. of a 10 per cent solution per 5 kg. body weight. The total dose should not exceed 6 ml. The course consisted of 3-4 intravenous injections at 3-4 day intervals.

The criteria for cure were based on the following factors: (I) The percentage reduction of the parasites in the circulating R.B.Cs as judged by the blood examination whenever possible made at each visit

to the clinic. (II) The clinical response as determined by the general condition of the animal and particularly the condition of the liver and spleen and the regain of appetite. (III) The improvement of the haemoglobin content as judged by the rapid test on the Tallqvists scale.

Materials and Methods

The results recorded are based on dogs presented for treatment with *B. gibsoni* infection to the Veterinary Clinic of the University of Ceylon, Peradeniya, and the Govt. Veterinary Hospital, Peradeniya from April, 1960 to April, 1961.

In each case wherever possible, when the disease was diagnosed, or suspected clinically, confirmation was obtained by the microscopic examination of blood. At this time the percentage of infected R.B.Cs., the haemoglobin level, and the temperature were recorded, and when the diagnosis was confirmed the computed dose of 'Spirotrypan' Hoechst was given intravenously and the owner was instructed to present the dog at 3-4 day intervals when the treatment and observations were repeated. Each case wherever possible was given 3 injections of 'Spirotrypan' and was seen on four occasions, when the blood was examined for the fourth time.

A few cases received a fourth injection. An animal was declared cured only if the appetite and temperature had come to normal and the haemoglobin level had increased considerably, and the incidence of the parasites in the R.B.Cs. was less than 0.1 per cent. However it was not possible to eradicate totally all the parasites from the red blood cells in some of the cases. Wherever necessary supportive therapy in the form of liver injections and parenteral or oral administration of iron and other haematinics were employed.

Results

The total number of dogs treated amounted to thirty two. Of these, the details of twenty cases are presented in table I. In the other twelve cases it was not possible to record the haemoglobin levels after each visit and to determine the percentage of infected R.B.Cs. before treatment and at each visit. In these cases, however, the disease was confirmed by microscopic examination and after the end of treatment was declared cured, only when all the clinical signs of the disease had disappeared and the blood was either negative for *B. gibsoni* or when the incidence of parasites was less than 0.1 per cent on microscopic examination. These 12 cases were simple uncomplicated cases which recovered at the end of the course of treatment. Hence the details of these are not presented.

In three of the cases (Nos. 3, 5 and 10) presented in table I there were very few parasites, always less than 0.1 per cent at the fourth visit, but all these dogs had recovered clinically and no relapses have been noted.

Out of the total 32 cases 2 dogs died (cases No. 12 and 19). In case number 12 death was due to cachexia and anaemia. The parasites were found to be multiplying in spite of the 'Spirotrypan' injection. In case number 19 death was found to be due to acute hepatitis and parasites were found in the red cells after death. Thus out of a total of 32 cases 30 dogs recovered completely, and of these, 27 cases showed no parasites at the end of the course of treatment.

Toxicity:—In all the cases studied there was no noticeable sign of toxicity in the dose employed. However, two dogs (case Nos. 4 and 7) which had received the injection rather rapidly showed mild transient signs of collapse, but these two dogs recovered in five minutes without any resuscitative measure. One of these dogs had previously received seven injections of Tryparsamide without result, for the treatment of *B. gibsoni* infection.

In atleast five dogs, the writers were aware of various degrees of mild extravasation of the drug during the intravenous injection due to various factors mainly due to the animal having struggled. There was mild transient oedema but suppuration or necrosis was not seen.

Six of the dogs (Nos. 3, 4, 10, 12, 13 and 19) had diseases which complicated *B. gibsoni* infection. Three of these (Nos. 3, 10 and 12) had varying degrees of nephritis and even though Spirotrypan was employed to cure *B. gibsoni* infection, this drug did not appear to be contra-indicated in nephritis. However, in these cases, antibiotics were given simultaneously to treat nephritis. It would appear that Spirotrypan is contra-indicated in cases of hepatitis (see case No. 19).

Discussion

The treatment of *B. gibsoni* infection has always been a difficult problem. Arsenicals have been employed and the results obtained with these have been variable. In our experience in Ceylon, Tryparsamide when used alone will cure 50-60 per cent cases clinically when 3-4 injections are administered, but the parasites do not disappear from the blood in some of these cases. This drug is comparatively safer than the other arsenical preparations, and though in most cases it has been given intravenously at the dosage rate of 1 gm./10 kg. body weight approximately, at 2-4 day intervals, it can also be given sub-cutaneously or intra-muscularly. In some cases up to seven injections have been employed without success. The great advantage with this preparation is its comparative safety. When Tryparsamide was combined with paludrine hydrochloride at the dosage rate of 0.05-0.2 gm. better results were obtained but in some cases there were either signs of vomition or jaundice.

TABLE I—Summary of 20 cases treated with 'Spirotrypan' Hoechst indicating the response to treatment.

Case No.	Clinical No. and Date	Age	Breed and Sex	First visit				Second visit				Third visit		Remarks
				Hb. %	Temp. °F	% R.B.Cs. infected	Dose of Spiro	Hb. %	Temp. °F	% R.B.Cs. infected	Dose of Spiro	Hb. %	Temp. °F	
1	4 U 4-1-60	8 yrs.	Labrador Male	70	103	1-5	6 ml.	75	101-4	Less than 0-1	4 ml.	75	101	This dog had been treated earlier with Tryparsamide without success. Animal cured.
2	69 U 8-2-60	4 yrs.	Labrador Male	75	102	3	8 ml.	—	—	Less than 0-1	—	—	—	This dog had been treated earlier with Tryparsamide and Tetracyclines without success. Animal cured.
3	383 U 29-7-60	1 yr.	Gr. Dane Male	70	104	2	6 ml.	75	102	0-8	6 ml.	—	102	This dog was found cured clinically at the 4th visit. A few parasites present; less than 0-1 per cent. This dog had nephritis and was treated subsequently with antibiotics.
4	384 U 29-7-60	3 yrs.	Gr. Dane x boxer Female	65	103	8	6 ml.	75	102	4	6 ml.	—	103	This dog was found to have heavy hookworm infection and was treated. After the administration of the first dose of Spirotrypan there was a transient sign of shock. However it recovered in the course of 5 minutes. It also had been treated earlier without success with 7 injections of Tryparsamide.
5	390 U 1-8-60	1 yr.	Alsatian Male	60	102-6	1-5	5 ml.	70	101	2	5 ml.	—	103	Dog clinically cured. A few parasites, (less than 0-1 per cent) present.
6	547 H 22-10-60	7 mon.	Alsatian Female	60	103-4	3	3 ml.	70	103-2	—	3 ml.	75	101	Cured. On examination of blood at 4th visit no parasites were seen.
7	555 H 23-10-60	2½ yrs.	Alsatian Male	—	104	4-2	3 ml.	—	104	—	3 ml.	—	101	Negative for blood parasites on examination of 4th visit. Signs of transient collapse during injection, but there was quick recovery.

8	556 H 23-10-60	7 yrs.	Alsatian Female	—	104	Moderate	3 ml.	—	103-6	—	3 ml.	—	101	—	Fourth visit Temp. 100-5; blood negative animal cured.
9	611 H 28-10-60	4 mon.	Alsatian Female	35	103-8	Moderate	3 ml.	75	103	—	3 ml.	80	102	—	Animal examined 3 days after the 3rd injection and found negative.
10	529 U 31-10-61	6 mon.	Alsatian Male	60	102-6	2	2 ml.	60	103	0-2	5 ml.	—	101-5	2-0	Animal cured clinically on 4th visit. Yet a few parasites present in blood. The animal also had severe ankylostomiasis and nephritis and was treated.
11	U 19-11-60	3 yrs.	Dachshund Male	80	102	1-2	3 ml.	80	102	—	3 ml.	80	102	—	Found cured on fourth visit, blood negative.
12	836 H 23-11-60	2½ mon.	Alsatian Male	45	104	Moderate	2 ml.	45	100	—	2 ml.	—	—	—	Dog died of acute anaemia and cachexia. It also had nephritis.
13	566 U 29-11-60	3½ yrs.	Dachshund Male	30	102-8	2	2 ml.	40	102	1-5	2 ml.	40	101	1-3	No parasites on the 4th visit. Dog was found to be heavily infected with hookworm and treated.
14	899 H 8-12-60	9 mon.	Alsatian Male	80	103	3	3 ml.	80	104	—	3 ml.	75	102-8	0-5	Negative for parasites on 4th visit.
15	957 H 6-12-60	6 mon.	Alsatian Male	70	103	4-2	2 ml.	75	102	4	3 ml.	80	—	—	Negative on 4th visit.
16	948 H 6-12-60	2 yrs.	Alsatian Male	80	103	High	3 ml.	60	102	—	3 ml.	—	—	—	This dog was not brought further for treatment.
17	964 H 8-12-60	6 mon.	Boxer Female	70	103	Moderate	3 ml.	75	102	—	3 ml.	80	—	0-2	Negative on 4th visit.
18	975 H 9-12-60	6 yrs.	Alsatian Male	70	103	3-4	3 ml.	70	102-8	—	3 ml.	75	101	3	Negative on 4th visit.
19	1063 H 16-12-60	6 yrs.	Spaniel Female	65	103	High	3 ml.	—	104	—	3 ml.	—	—	—	The dog died of acute hepatitis.
20	19 U 6-3-61	4 yrs.	Boxer Male	80	103-4	13	4 ml.	45	102	13	4 ml.	60	102	5	The dog was treated also for acute nephritis and clinically cured. But it was not possible to examine the blood after the 3rd injection as it was not brought to the clinic again.

When neorasphenamine B.P.C. (neoarsenobillon; N.A.B., M. & B.) was given intravenously at 0.1 gm./10 kg. body weight at 3 — 4 day intervals, it has given upto 70 per cent recoveries. However this drug has been found to be rather toxic and perivenous infiltration was found to cause oedema, necrosis or suppuration.

All the above percentages have been judged in cases that indicated recovery on clinical grounds only.

However, it has been found that when a 10 per cent solution of 'Spirotrypan' (Hoechst) was used intravenously at 1 ml./10 kg. body weight for 3 — 4 days at 3 — 4 day intervals it gives a higher rate of recovery than any other drug employed hitherto. Further, it has been found to be less toxic than neorasphenamine. In all these cases recovery has been judged both on clinical grounds, and on the basis of microscopic examination of blood.

As to why it is not possible to obtain a 100 per cent recovery is difficult to explain. The possibility of the existence of an arsenic resistant strain has been suggested by some (Imperial Veterinary Research Institute Mukteswar report 1934-35). The existence of two or more species of *Babesia* or the presence of other complications in the clinical cases so treated which might have been overlooked are probably some of the factors. Recent investigations by one of us (P.S.) in experimentally infected dogs made in the laboratory indicate that the likely reason for the poor response to arsenic therapy is that the arsenicals are active against only one or more stages of the parasite. These investigations have indicated that there are several stages of the parasite in the dog. Arsenicals do not appear to be very effective when the parasites are actively multiplying. The writers have noticed that whatever line of treatment is employed the recovery is always slow. The dramatic response obtained after a single injection of an arsenical (Salvarsan) claimed by the earlier writers (Symons and Patton 1912) is probably mainly due to the fact that arsenic injection acted as a tonic, as microscopic examination at this stage does not show any marked reduction in the number of parasites in the R.B.Cs.

When one considers the marked clinical improvement obtained when 'Spirotrypan' is given, it is unlikely that the action of the arsenical is merely that of a tonic. The administration of liver or iron injections alone does not produce any noticeable clinical improvement.

Even though the results reported herein with 'Spirotrypan' are superior to those obtained with the use of other arsenicals used earlier, it is still felt that the ideal chemotherapeutic agent for the treatment of *B. gibsoni* infection is yet to be found. It is possible that an arsenical

would have to be combined with one or more other drugs such as antimalarials, to obtain good and quick response in these infections.

Summary

1. *B. gibsoni* infection is widely prevalent in dogs in Ceylon.
2. 'Spirotrypan' Hoechst when given as a 10 per cent solution by slow intravenous injection at 3 — 4 day intervals for 3 — 4 days, appeared to be superior to other arsenicals employed in the treatment of this infection.
3. The dosage employed was 1 ml. per 5 kg. body weight, not exceeding a total dose of 6 ml. per dog per injection.
4. Of the 32 dogs treated 30 were cured clinically and in, 27 no parasites were seen in the blood films. Two of the dogs which had very heavy infections did not respond to treatment and died. One of these had acute hepatitis while the other was very anaemic.

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Abstracts

Egg Quality Declines:—A slow decrease in interior quality of eggs occurs throughout the laying year, but the greatest decline occurs after the first two months of lay. This has been reported by C. W. Pope and his co-workers of the Louisiana University. Working with a production strain of New Hampshires, they found a high rate of production did not seem to affect the interior quality of eggs. Shell quality decreased throughout the laying period, and the greatest decline began in February and continued to drop at a significant rate during the rest of the laying period. This seemed to coincide with the rise in temperature occurring with the warmer weather. They also observed that hens which laid at a low rate had a tendency to produce eggs of lower quality than hens laying at a higher rate. (*Poultry Sci.*, **39**, No. 6).

B. PANDA.

How Chicks Break Out:—Chicks use a combination of their muscles and instinct to break out of eggs at hatching time, according to

Prof. Harvey Fisher of Southern Illinois University. When a chick reaches full development inside the eggs, its brain signals a muscle which stretches from the back of its head to the nape of its neck. This muscle pulls the head back and forth in a pecking motion which cracks the shell, allowing the chick to get out. (*Poultry Digest*, May, 1961).

B. PANDA.

Histochemical Changes in Mouse Skeletal Muscle associated with *Trichinella spiralis* Infection:—The author, E. B. Beckett, Department of Histology, University of Liverpool has studied the disintegration of infected muscle fibres in the gastrocnemii of mice infested with *T. spiralis*. From muscle pieces, fixed briefly in cold neutral normal saline, frozen sections were cut, and were used to demonstrate aminopeptidase by the technique of Nachlas, Crawford and Seligman (1958). The results indicate that *T. spiralis* larvae cause chemical breakdown, and probably depletion, of host muscle proteins, and it is possible that aminopeptidase is involved. It is known that the larvae can incorporate labelled amino acids administered to the host, although it is not certain that these amino acids are first incorporated into the host protein. Other workers have shown that *Trichinella* larvae possessed no tryptic activity. (*Biochem. Journal*, Vol. 78, Feb., 1961).

B. PANDA.

A Rapid Method of Fixing Delicate Protozoans:—A procedure for a rapid method of fixing delicate protozoans has been reported by B. R. Hamilton and D. J. Campbell, Ontario Veterinary College, Canada. The report indicates that a loopful of ingesta containing motile protozoans is placed near one end of a slide. To this is added a drop of blood, and these materials are mixed, smeared across the slide, and allowed to dry. This produces fixation with minimal distortion. Staining is carried out in the usual manner, using Giemsa's or Wright's stain. The latter appears to give better definition of flagella. Fresh blood is required for fixation and is obtained by puncturing a finger as in blood tests. This, of course, is a disadvantage but is compensated by the satisfactory results obtained. In addition the presence of blood cells of known size, on smears processed by this method is useful for comparative measurements of the organisms under examination. This method, according to the authors, can also be used for fixing and staining protozoans from cultures or other media. (*Poultry Science*, Vol. 40, 1132, 1961).

B. PANDA.

Haemorrhagic Lesions in Chickens:—For approximately a decade a malady known as "Haemorrhagic Syndrome" has affected chickens in the United States of America. Various etiological factors have been

ascribed for the causation of this disease in chickens. Recent research conducted by G. Schumaker, B. Panda and co-workers at the University of Maryland has indicated that two species of Aspergilli—*A. flavus* *oryzae* (NRRL A 4018 b), and *A. chevalier* (NRRL A 4020) are responsible to produce lesions indistinguishable from typical haemorrhagic syndrome, when fed orally. Haemorrhages were seen in leg muscles, breast muscles, liver, spleen, kidney and heart. In addition, yellow or pale bone marrow was noticed, in 3 out of 5 birds in each mold-fed group. (*Poultry Science*, Vol. 40, 1132-1134, 1961).

B. PANDA.

Studies in Immunochemistry II. Physico-Chemical properties of specific polysaccharides obtained from different serotypes of *Corynebacterium renale*:—By DHANDA, M. R., PANDA, S. N., MURTHY, P. S. N. and SEKARAH, P. C. (1960), *Ind. J. Path. Bact.*, 3 (4), 222-226.

From strains of *Corynebacterium renale* representing each of three serological types (Panda and Dhanda, 1959), polysaccharides were isolated after disintegration of acetone-dried organisms with phenol or alkali. The materials were found to carry the specificities on which the serological classification of the species is proposed to be based. Analyses showed some unusual features, rhamnose being conspicuously absent in all the materials which, however, contained arabinose. All the polysaccharides from the three serotypes were somewhat similar in monosaccharide composition, which resembled the pattern reported from preparations of cell-walls of *C. renale*, indicating the probable site of their localisation on the intact cells of *C. renale*.

S. N. PANDA.

Studies in Immunochemistry III Immunobiological Characterisation of Polysaccharides obtained from different serotypes of *Corynebacterium renale*:—By DHANDA, M. R., PANDA, S. N. and SEKARAH, P. C. (1960), *Ind. J. Path. Bact.*, 3 (4), 227-236. (Authors' summary).

The serological specificities of polysaccharides obtained from representatives of each of the three serotypes of *C. renale* were examined by the lytic modification of indirect bacterial haemagglutination and the corresponding inhibition tests. Absolute type specificity was found by this method. By agglutination inhibition test, the polysaccharides were shown to be antigens located on the surface of intact cells on *C. renale*. They were relatively poor antigens, when isolated, but their antigenicity could be enhanced by combining with lipoprotein or by injection of antigen-coated erythrocytes or heat killed streptococci. It was considered that the polysaccharides bear the specificity of somatic antigens on which the serological grouping of the species is proposed to be based. Polysaccharides obtained from *C. equi*, *C. ovis* and *C. pyogenes*

failed to cross react to significant titre by antisera produced against the three serotypes of *C. renale* though polysaccharides of *E. monocytogenes* reacted to low titre.

The polysaccharides proved non-toxic for mice and non-pyrogenic to rabbits, but they exhibited interesting pharmacodynamic properties on inoculation into mice with special reference to circulation in the kidneys.

S. N. PANDA.

News and Views

A Veterinarian as an Ambassador:—The Ambassador to the United States and to the United Nations from Senegal, Africa, is a Veterinarian.

Dr. Ousmane Soce Diop, holds his Veterinary degree from Alfort University, France and the University of Paris. His thesis concerned experiments in the use of cobra venom in cancer research. He is in addition an author, poet, magazine editor, Mayor of the City of 60,000, and Deputy to the French community of his nation. He has served in the French Senate for 15 years. He has written two novels, one of them a literary prize winner, and a volume of poetry.

New Jersey Veterinary Medical Association invited him to address their convention in March last and he was enrolled as an honorary member of the Association. (*J.A.V.M.A.* dated 15th June 1961).

* * *

A Remarkable Speech at "Neutral Summit":—Mrs. Sirimavo Bandaranaike, the talented Prime Minister of Ceylon, gave vent to words that should find an echo in the hearts of all decent people. She said "I have come to this Conference (at Belgrade) also as a woman and a mother. I do not believe for one moment there is a single mother in the world who can bear to contemplate the possibility of her children being exposed to atomic radiation and slow and lingering death". Man may not care to listen but God does. Let us have faith in Him.

* * *

Professor J. M. Guerin, The great French Veterinarian who attained world-wide fame for his evolving the B. C. G. vaccine against Tuberculosis, in association with Professor Calmette, died in Paris at the age of 89. He is said to have worked for 36 years in perfecting the B. C. G. vaccine, which was first used on children early in this century. Such is the single-minded devotion to Research in other countries. Where does India stand in this matter? "Where" answers the echo!

Tetracycline Plant:—The Pfizer Antibiotic Plant at Chandigarh, Punjab, the first to make tetracycline from Indian basic raw materials is now complete and production has started. Pfizer is the first to start basic manufacture of tetracycline in the country and this plant is the first of its kind in India. Designed to make the oxytetracycline and tetracycline required for the production of Terramycin, Synermycin and Tetracyn, the Pfizer plant will effect a considerable saving of valuable foreign exchange.

Association News

The Indian Veterinary Association

THE RESERVE FUND

An event of great importance to the Veterinary profession in India, took place in August 1924. It was the birth of the *Indian Veterinary Journal*. Circumstances were positively hostile and yet the profession took that bold step and the lion-heartedness of the early pioneers cannot be too highly praised. Ever since then, the *I.V.J.* has toiled hard under very adverse circumstances. The *I.V.J.* lives to-day so that the profession could prosper and Science advance.

And now the hat is sent round so as to place this journalistic venture on a sounder and firmer basis. The *I.V.J.* is a priceless possession of our profession. It has placed the Indian Veterinary Surgeon, on the map of the scientific world. Just pause and ponder over, how much we owe to the *Journal* both individually and collectively. Is it too much then to expect the profession to respond to the appeal issued for a Reserve Fund? We hope not.

Some enquiries have come to us asking us to indicate the extent of help expected. We do not want to put the members of the profession to any undue financial strain in these hard days. At the same time, a certain amount of sacrifice is needed to make this fund a great success, so as to make it worthy of our profession. So we feel that the following scale of contribution will be quite reasonable. It could even be paid in two or three instalments.

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All others	...	Rs. 25/- and above.

It is our ambition that no Veterinary Surgeon in this land should remain without contributing to this fund. Brick by brick, this great edifice of ours should begin to rise higher and higher. On the success

or otherwise of this venture, the solidarity of our profession depends. "Pay and Prosper" is the slogan we pass on to the members of our profession.

Jai Hind!

T. VINAYAKA MUDALIAR,
Editor, *The Indian Veterinary Journal*

Cheques, money orders and all correspondence should be addressed to the Editor, *The Indian Veterinary Journal*, Madras-17.

Review

The Veterinary Annual—Second Issue (1960):—By W. A. POOL, M.R.C.V.S. Price 38s. 6d. John Wright & Sons, Ltd., Bristol-4, England. Pages 359. 32 plates.

The second issue of the Veterinary Annual provides within its brief compass an excellent fare on the activities and achievements of the profession in its multifarious fields subsequent to the period covered in the first issue (1959).

The highly informative chapters, the exhaustive references under each and the illuminating illustrations are bound to provide stimulus to the members of the profession in their day-to-day work. It is becoming increasingly clear that the Veterinary Annual will come to stay as an integral part of the world Veterinary literature.

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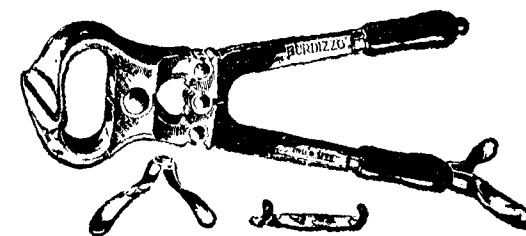
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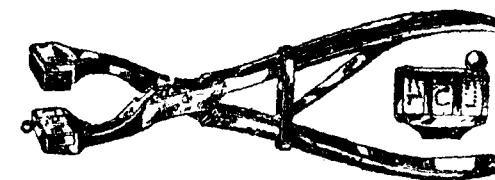
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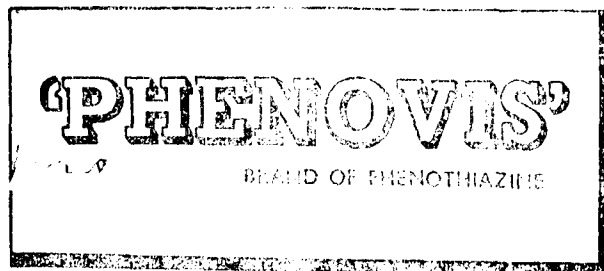
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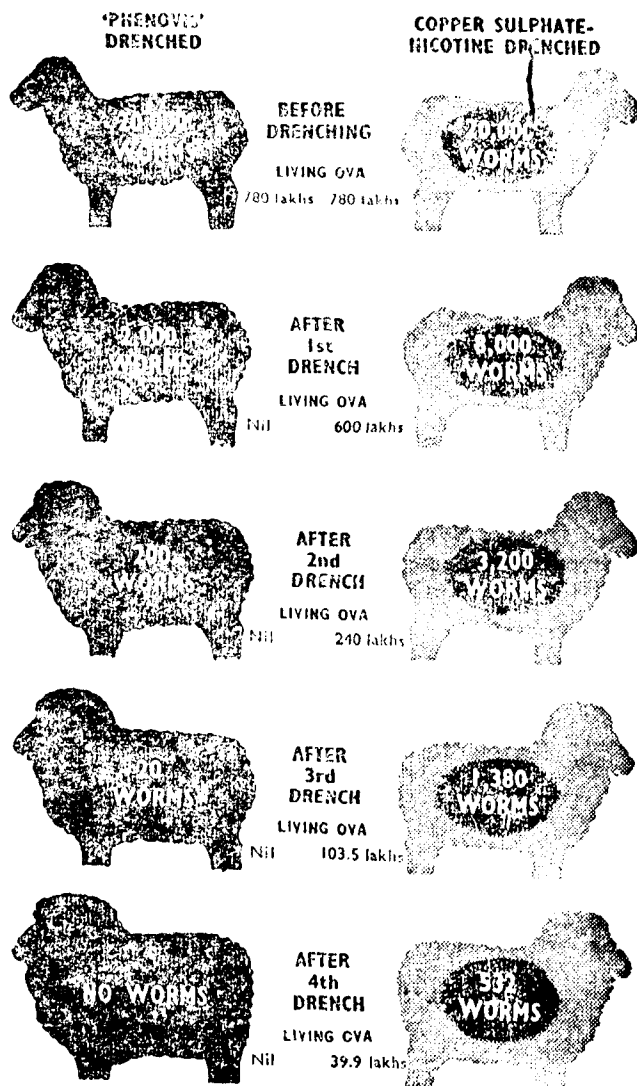


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