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OBSERVATIONS
ON THE
MIGRATION OF WARBLE LARVÆ
THROUGH THE TISSUES

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Honourable MARTIN BURRELL,
Minister of Agriculture.

SIR,—I have the honour to submit to you a report with regard to Observations on the Migration of Warble Larvae through the Tissues, by S. Hadwen, D.V.Sc., Pathologist, Veterinary Research Laboratory, Agassiz, B.C., and E. A. Bruce, V.S., Inspector in charge of Meat Inspection, Vancouver, B.C., and request that it be printed as Bulletin No. 22.

I have the honour to be, sir,

Your obedient servant,

F. TORRANCE,
Veterinary Director-General.

OTTAWA, 8th May, 1916.

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OBSERVATIONS ON THE MIGRATION OF WARBLE LARVÆ THROUGH THE TISSUES.

(BY S. HADWEN AND E. A. BRUCE.)

The first part of this paper deals with experiments with larvæ introduced under the skin; the second with the examination of gullets infested with warble larvæ; and the last part with the probable course of the larvæ after leaving the gullet, and the anatomical structures involved.

EXPERIMENTAL INTRODUCTION UNDER THE SKIN OF LARVÆ TAKEN FROM THE GULETS OF CATTLE.

This experiment was undertaken to ascertain what channels the larvæ passed through, on their journey to the back. In previous experiments it had been shown that larvæ, removed from a gullet, could be introduced under the hide of another animal, and that they would find their way to the back. Though this fact had been established, it was desirable to repeat the experiment, and if possible to discover the path taken by the parasite, and find it before it had reached the back.

PROTOCOLS.

1914, November 17.—Five warble larvæ were dissected out of a cow's gullet. Four of these were about 1 cm. long, and one 1.5 cm. in length. These larvæ were slipped under the hide on the outside of a calf's knee. The calf was three weeks' old.

December 7.—Two of the larvæ had punctured the skin on the calf's back, 2 to 3 inches behind the posterior angle of the scapula. One of these was squeezed out and measured 1.4 cm. in length. The calf was killed. The leg was skinned and a gelatinous patch was found at the point where the larvæ had been introduced, viz., on the outside of the knee, at a point over the tendons of the extensor muscles. This gelatinous track was followed and extended in towards the radius, passing round it posteriorly, then going upwards under the elbow joint. The track was plainly visible in the white fibrous tissue. From the inside of the elbow joint it passed up under the scapula, and a larva was found about two-thirds of the way up this bone, near its posterior border. That is to say, under the subscapularis and above the serratus magnus (plate II, fig. 7). This larva was 1.5 cm. in length. The reason why the track of the parasite was so plainly visible, was the fact that no special care was taken in removing the larvæ from the gullet; hence these became contaminated with bacteria and naturally carried infection with them wherever they went, thus leaving a plain trail.

In a previous paper, good reasons were given for the larvæ not becoming encysted or destroyed in the body. They kept constantly moving, and though they carried infection with them, causing more inflammation than they would have done if free from bacteria, the tissues were powerless to arrest their progress. Four other experiments of the same nature were unsuccessful; the larvæ were weak, having been kept for too long a period before being introduced beneath the skin of another animal. In the successful experiments, those mentioned in the previous report and the one just described, the larvæ were introduced into a fresh host as soon as possible after removal (about half an hour). In some of the experiments which failed they had been removed for much longer periods, and had lost their vitality.

It would appear that a mistake can easily be made in keeping the larvæ too long or at too high a temperature. In several cases larvæ died when placed in an incubator at 37.5° C. after a few hours. There was no impairment of vitality in larvæ kept for three hours in a cool place. With others kept ten hours in a vial at body temperature, bacterial action had been very destructive. The larvæ were dead and putrefaction had taken place. The conclusions to be drawn from these experiments are that the larvæ travel through fibrous tissue, and that if they are removed from the gullet at the proper time, go directly to the back. In 1914 warble larvæ had perforated the backs of a number of cattle on December 15; their average length was about 1.2 cm. This shows that the experiment just described was undertaken at the right period.

EXAMINATION OF INFESTED GULLETS, DURING WINTER OF 1914-15.

The examinations were made at regular intervals throughout the winter, the object being to discover where the larvæ were situated and to determine their method of migration, where they came from and where they went to. A table has been made out for each month, giving the number found, their position in the gullet and their direction, whether ascending or descending.

A short summary follows each table. The gullets were all secured from Alberta cattle.

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TABLE FOR JANUARY, 1915

Date.	Number of infested gullet.	Length of gullet.	Position of larvæ in gullet measured from pharyngeal end	Number of larvæ ascending	Number of larvæ descending.
January 25.	7	99 cm.	1 " 33 cm	0	1
			2 " 51 "	2	
			1 " 52 "		1
			1 " 57 "		1
			1 " 59 "		1
			1 " 60 "	1	
			2 " 66 "		2
			1 " 69 "	1	
			1 " 72 "		1
			1 " 91 "	1	
		1 " 96 "		1	
"	8	105 cm.	1 " 15 "		1
			1 " 33 "	1	
			1 " 48 "	1	
			1 " 50 "		1
			1 " 74 "		1
		1 " 75 "	1		
"	9	93 cm.	1 " 19 "	1	
			1 " 48 "	1	
			1 " 56 "	1	
			1 " 61 "		1
			1 " 63 "		1
			1 " 66 "		1
			1 " 78 "		1
			1 " 81 "		1
			2 " 87 "		2
					1 " 90 "

The three infested gullets contained an average of 10 larvæ per gullet. Twelve were ascending and eighteen were descending. The average distance from the pharyngeal end was 55 cm., and the average length of the larvæ was 1.3 cm.

February

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1.5 cm.

TABLE FOR FEBRUARY, 1915.

Date	Number of infested gullet.	Length of gullet.	Position of larvæ in gullet measured from pharyngeal end.	Number of larvæ ascending.	Number of larvæ descending.
February 2.....	10	87 cm.	1 " 32 cm. 1 " 48 " 1 " 67 "	1 7	1
"	11	90 "	1 " 39 " 1 " 45 " 1 " 88 "	1 1 1
"	12	81 "	1 " 51 "	1
"	13	83 "	1 " 53 " 1 " 60 " 1	1
"	14	78 "	1 " 35 " 1 " 78 "	1 1
"	15	90 "	1 " 30 " 1 " 51 " 1 " 79 "	1 1 1
"	16	?	1 " 40 "	1
" 18	17	99 "	1 " 43 " 1 " 63 "	1 curved.
"	18	90 "	1 " 72 "	1
"	19	96 "	1 " 69 " 1 " 75 "	1 1
"	20	87 "	1 " 45 " 1 " 63 "	1 1
"	21	81 "	1 " 45 " 1 " 63 " 1 " 66 "	1 1 1

The twelve infested gullets contained an average of 2.5 larvæ. Eleven were ascending and thirteen descending, one being curved. The average distance from the pharyngeal end was 66.3 cm., and the average length of the grub 1.5 cm.

TABLE FOR MARCH, 1915.

Date	Number of infested gullet.	Length of gullet.	Position of larvæ in gullet measured from pharyngeal end.	Number of larvæ ascending.	Number of larvæ descending.
March 6	22	?	1 " 43 cm.		1
"	23	?	1 " 60 " 1 " 63 "		1 1
"	24	?	1 " 72 "		1
" 15	25	90 "	1 " 75 "		1
"	26	90 "	1 " 72 "		1
"	27	90 "	1 " 79 "		1
" 24	Sixty-one gullets examined.		One dead larva found, 15 cm. from paunch end.		

The six infested gullets contained, on the average, 1.1 larvæ per gullet. All these were descending. The average distance from the pharyngeal end was 66.3 cm., and the average length of the grubs 1.5 cm.

In summarizing these observations it is clear that the larvæ found in the gullets are numerous in December and decrease gradually until they disappear, about the middle of March. We were somewhat disappointed in not being able to show a definite migration from one end or the other of the oesophagus. However, the last larvæ were found in the lower end, but in small numbers. Professor Carpenter and his associates had a very similar experience.

A source of error which Professor Carpenter has evidently overlooked is the difference in the life-history of *H. lineatum* and that of *H. bovis*. This applies also to our own work, seeing that *H. bovis* is a much later fly than *H. lineatum*, and no doubt their larval stages overlap. This point is well illustrated in this season's work. Two ♀s of *H. lineatum* were captured out of doors as early as April 15: whereas *H. bovis* had not made its appearance a full month later. Hence the larvæ next year must be of necessity at least a month later than those of *H. lineatum*. Thus the two life-histories overlap. Several larvæ were found at the junction of the oesophagus and the paunch: here the muscle fibres are widely separated and have a radiating arrangement. This is undoubtedly the place where the larvæ would start on their journey to the back. They were here found pointing in different directions. At this point there is a reflection of the pleura over the end of the gullet, beneath which they could readily pass. On finding that the larvæ were disappearing from the oesophagus, we next directed our attention to tracing them in their subsequent migrations.

Course of larvæ.—The following facts in connection with the course of the larvæ have been verified by observation:—

- (a) The larvæ are found in the submucosa of the oesophagus and gradually work their way towards the diaphragmatic end of the oesophagus.
- (b) They may follow the posterior borders of the ribs under the pleura.
- (c) They may enter the neural canal.
- (d) Evidences that the posterior foramen is the means of ingress and exit have been noted.
- (e) Finally the larvæ follow connective tissue closely.

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At the time that the larvæ were leaving the oesophagus a lack of subjects for examination proved a serious handicap; however, with the foregoing facts established, it is possible from an anatomical standpoint to trace with a fair degree of accuracy the course of the larvæ from the oesophagus to the back. In cattle the oesophageal opening of the diaphragm is situated some 4 or 5 inches below the eighth thoracic vertebra, and is formed by the right crus of the diaphragm, which divides into two branches, uniting again below the opening. The oesophagus is partly covered by pleura, and it may therefore be assumed that the larvæ pass under the pleura; once there they may follow one of two courses, viz., up the crura or across the diaphragm. The latter course will now be considered, as it is the only one that we have direct evidence about. The larvæ follow the general direction of the muscle fibres of the diaphragm, downwards and outwards, until they gain the cartilages of the ribs. The costal attachment, of the diaphragm extends in a nearly straight line from the upper fourth of the last rib to the junction of the eighth rib with its cartilage and along the latter to the sternum. It would therefore appear that the larvæ follow the ribs in this region. Proceeding along the posterior border of the ribs in company with the vessels which are ventrally sub-pleural, they pass with the vessels between the intercostal muscles. Upon reaching the upper part of the intercostal space some larvæ may pass directly into the canal through the posterior foramen and possibly entrance may also be made through the intervertebral foramen which is smaller. No evidence of the latter has, however, been found, while in the case of the former evidences of larval passage have been noted. The natural course of the larvæ upon reaching the upper part of the intercostal space, would be to pass between the external intercostal and the levator costarum, then under the longissimus, passing between that muscle and the transversalis and eventually gaining the skin.

That the larvæ may pass up the crura of the diaphragm upon leaving the oesophagus appears likely—such a course would be considerably shorter than the course up the ribs, and possibly may be the route most commonly followed. No insurmountable difficulties would be encountered, beyond the possibility of having to pass between the fibres of the sublumbar muscles, which at this point are comparatively thin; further, the neural canal could be easily reached, and exit made through the posterior foramen, either further up or down the spine. The only evidence we have of such a course being followed is the actual finding of larvæ in the neural canal. The larvæ are situated on the thoracic side of the crura of the diaphragm, and from here must pass to the lumbar region, either down the canal or along the fibrous tissue between the muscles.

Professor Carpenter found larvæ just outside the muscular coat of the gullet, in the thoracic region, close to the diaphragm, which finding supports our contention.

In one of the animals examined, we found a distinct track along the posterior border of the ninth rib. This track was of a green colour and was plainly visible, leading up to the spinal canal, through the posterior foramen. The fatty tissues surrounding the cord in the canal was tinted the same colour. Altogether eight larvæ were found in the spinal canals of about twenty animals examined. The larvæ were encountered in the dorsal region and four of them measured respectively, 1.2, 1.2, 1.0, and 1.4 cm., giving an average of 1.2 cm. Two were found emerging from the posterior foramen. On the same date those found in the gullet averaged 1.5 cm. and a number of larvæ found just under the skin of the back were the same size.

The larvæ were found in the areolar tissue which surrounds the dura mater: whenever we found one there were evidences of greenish pigmentation and of a gelatinous infiltration at different points along the cord. The fat also exhibited some curious grey degenerate areas, so that it is evident that the larvæ cause some irritation. Moussu informed one of us (S.H.) that he had seen nervous symptoms in cattle which he attributed to this cause. The occurrence of these inflammatory tracks was noted, even where no larvæ were found.

Drawings have been prepared by one of us (E.A.B.), illustrating the anatomical side of the question. Red lines indicate the probable course taken by the larva. The task has been a difficult one owing to the fact that the works on veterinary anatomy do not describe the parts involved in sufficient detail.

In summing up, the following points have been established:—

- (1) The larvæ follow connective tissue closely.
- (2) In British Columbia they may perforate the skin of the back as early as December 15.
- (3) The latest date on which larva were found in the gullet was March 15.
- (4) Larvæ found in the oesophagus, spinal canal, and subcutaneous tissue, all had about the same dimensions; signs of their migration through the intervening tissue were also found, proving that the process is continuous.

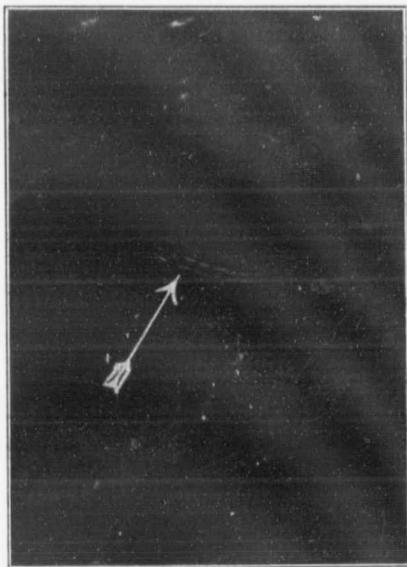


FIG. 7.

EXPLANATION OF FIGURES.

Fig. 1. Explains itself.

" 2. Section through eighth thoracic vertebra.

" 3. Section through tenth thoracic vertebra.

" 4. Inner surface of ribs.

" 5. Outer surface of ribs, external muscles removed.

" 6. Section through spine.

" 7. Warble larvæ photographed between the subscapularis and the serratus magnus.

Nos. 2 to 6, inclusive, are all drawings of the same piece of meat.

"A" Skewer, showing posterior foramen.

"B" Skewer, showing intervertebral foramen.

Direction of skewers shown as they appeared when pushed well into foramen.

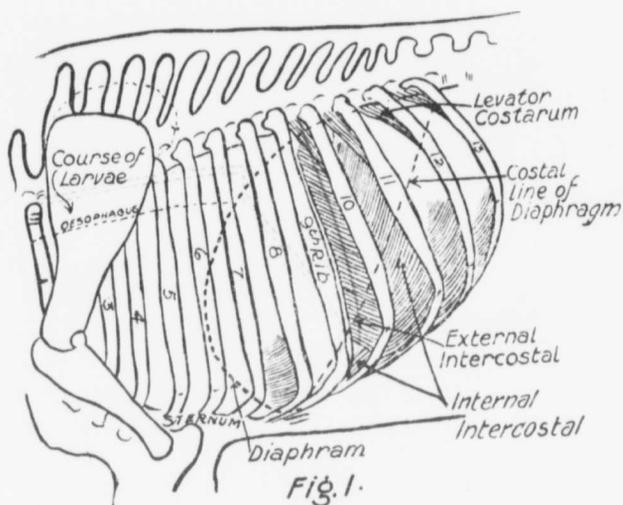


Fig. 1.

Section through 8th Vertebra

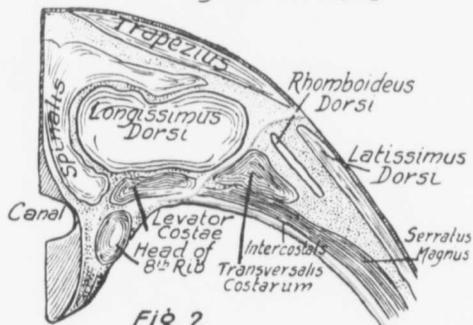


Fig. 2.

Section through 10th Vertebra

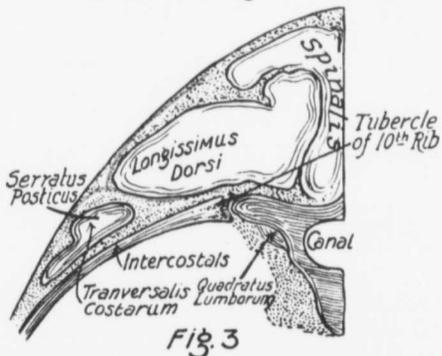


Fig. 3

Inner Surface of Ribs

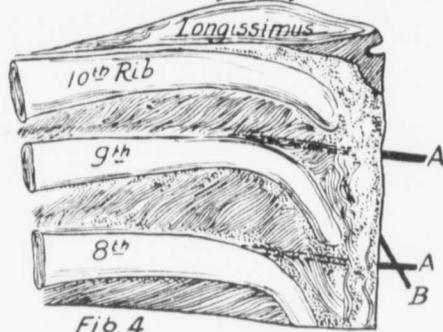
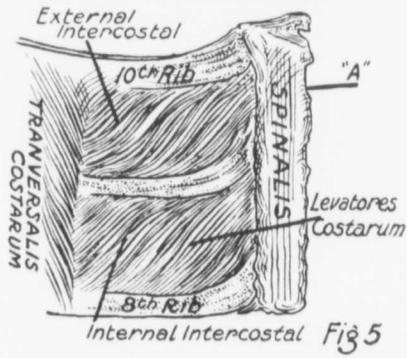


Fig. 4

External Muscles Removed



Internal Intercostal Fig 5

Section through Spine

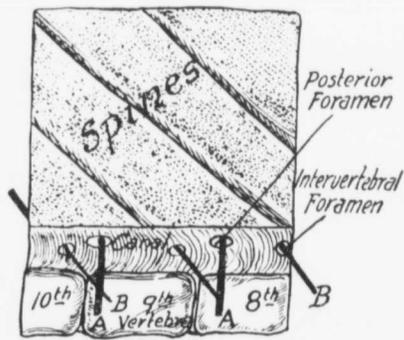


Fig. 6