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A STATISTICAL METHOD FOR COMPARING WORM BURDENS IN TWO GROUPS OF SHEEP

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ABSTRACT

GROENEVELD, H. T. and REINECKE, R. K. A statistical method for comparing worm burdens in two groups of sheep. *Onderstepoort J. vet. Res.*, 36 (2), 285-298, (1969).

In groups of experimentally infested sheep, worm distributions are markedly skew. In controlled anthelmintic tests, the worm burdens of treated and control sheep have different distributions and this invalidates the use of transformations.

Five experiments are described, of which the first three describe the evolutionary steps taken to find a suitable method for interpreting the data. A non-parametric method was evolved and the last two experiments demonstrate the use of this test to interpret the results. The entire method is explained and tables are included which simplify its use for biologists with no statistical training.

INTRODUCTION

For some years, experiments have been carried out at Onderstepoort in an attempt to produce uniform worm burdens in sheep. The optimal conditions for the infestation of worm-free sheep with infective larvae of their common gastro-intestinal nematodes have been described (Reinecke, 1963, 1966 a, b, 1967, 1968; Reinecke & Anderson, 1967; Reinecke, Horak & Snijders, 1962; Reinecke, Snijders & Horak, 1963). Normal distribution, however has not been achieved.

The assessment of an anthelmintic's efficacy depends on a comparison between the worm burdens of a group of treated sheep with those of an untreated control group. In common with most other workers, we have done this by comparing the mean worm burdens of the two groups, and have expressed the results in terms of percentage reduction. This method only takes into account a reduction of the mean worm burdens of a flock; it makes no allowance for the erratic effect of the anthelmintic on the worms in individual sheep. As the worm population before treatment is unknown the actual reduction in any individual sheep cannot be determined. Moreover, the individual worm burdens of the treated sheep, arranged in ascending order, cannot be directly compared with the controls in the same order. If this was the case, the consistency of reduction of worm population could be determined.

Egerton, Ott & Cuckler (1963) have shown that sheep with natural infestations have worm distributions that are markedly skew. They stated: "Statistical tests of significance such as 't', 'Z' and 'F' tests, estimates of confidence limits, potency ratios and others, are based on normally distributed populations. The validity of such procedures, therefore, is dependent upon the normalcy of the experimental data". Our experiments with artificial infestations have repeatedly confirmed this observation.

In an attempt to comply with the requirements of parametric methods, Egerton *et al.* (1963) used a logarithmic transformation. It appears, however, that the worm burdens of controls and treated sheep have different distributions and this, in our view, invalidates the use of transformations.

In attempts to solve this problem five experiments were carried out. The first three depict the evolutionary steps taken to find a suitable method for interpreting the data. A non-parametric method was ultimately chosen and the last two experiments were designed and carried out to demonstrate its use to interpret the results.

EXPERIMENT 1

Materials and Methods

1. Eighteen weaned Dorper (Dorset Horn × Black Head Persian) sheep, born, reared and maintained worm-free were used. They were infested with infective larvae of *Oesophagostomum columbianum* (Curtice, 1890) using the methods described by Reinecke (1968).

2. The experimental design is summarized in Table 1.

3. After slaughter the worms were recovered and total worm counts were carried out by the methods described by Reinecke (1967).

4. The larval stages were identified according to the descriptions given by Veglia (1923), the moults being classified with the previous larval stage (Reinecke, 1968).

Results

These are summarized in Table 2.

The parasites recovered from Sheep 1 killed on Day 0 indicate the stages of development of worms on the day of treatment. Usually this worm burden is not included with those of the control group when estimating the mean, because fewer worms are recovered from it than from the controls killed two or three days later, and the proportion of the various larval stages differs from those in animals slaughtered subsequently (Reinecke, 1968). In this case, Sheep 1 had only nine less worms than Sheep 4 killed three days later and the proportion of the larval stages in it compared favourably with those in the other controls.

If the means of the total worm burdens of the three groups are compared in an analysis of variance procedure, a highly significant F-value is obtained.

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TABLE 1. — *Experiment 1. Experimental design*

Day	No. of infective larvae of *O. columbianum* dosed to each sheep	
—27		80
—18		80
—14		80
—11		80
— 9		80
— 7		80
— 5		150
— 3		150
— 1		150
Total		1,010
0	Slaughtered Sheep 1, Day 0 Control Treated Sheep 7—12 inclusive with haloxon at 100 mg/kg intraruminally. Treated Sheep 13—18 inclusive with haloxon at 200 mg/kg intraruminally.	
+ 2	Slaughtered Sheep 2 & 3, Day + 2 controls Slaughtered Sheep 7, 8 & 9, treated on Day 0 at 100 mg/kg Slaughtered Sheep 13, 14 & 15, treated on Day 0 at 200 mg/kg	
+ 3	Slaughtered Sheep 4, 5 & 6, Day + 3 controls Slaughtered Sheep 10, 11 & 12, treated on Day 0 at 100 mg/kg Slaughtered Sheep 16, 17 & 18, treated on Day 0 at 200 mg/kg	

TABLE 2. — *Number of* Oesophagostomum columbianum *recovered at autopsy*

Group	Sheep No.	Stage of Development			Total
		L_3*	L_4	5	
Controls					
Killed on Day 0	1	30	30	59	119
Killed on Day +2	2	65	63	73	201
Killed on Day +2	3	20	118	46	184
Killed on Day +3	4	24	95	9	128
Killed on Day +3	5	50	145	20	215
Killed on Day +3	6	3	69	80	152
	Mean	32.0** s.d. 22.2	86.6 s.d. 29.2	47.6 s.d. 28.6	166.0 s.d. 39.5
Treated on Day 0 with haloxon at 100 mg/kg					
Killed on Day +2	7	10	50	0	60
Killed on Day +2	8	22	23	0	45
Killed on Day +2	9	26	80	8	114
Killed on Day +3	10	15	59	4	78
Killed on Day +3	11	10	53	0	63
Killed on Day +3	12	64	62	0	126
		24.5 s.d. 20.3	54.5 s.d. 18.9	2.0 s.d. 3.3	81.0 s.d. 32.2
	Mean Reduction	23.4%	37.1%	95.8%	51.2%
Treated on Day 0 with haloxon at 200 mg/kg					
Killed on Day +2	13	72	9	0	81
Killed on Day +2	14	20	22	0	42
Killed on Day +2	15	64	34	4	102
Killed on Day +3	16	3	37	0	40
Killed on Day +3	17	13	25	0	38
Killed on Day +3	18	43	45	0	88
	Mean	35.8 s.d. 28.3	28.6 s.d. 12.7	0.6	65.2 s.d. 28.4
	Mean Reduction	—	67.0%	98.7%	60.7%

*L_3 — third stage larvae
L_4 — fourth stage larvae
5 — fifth stage
**s.d. — standard deviation

TABLE 3. — *Experiment* 2. *Experimental design. Larval viability controls*

Day	No. of infective larvae dosed	
	Oesophagostomum columbianum	*Dictyocaulus filaria*
	Sheep 19 larval viability control Day-18 to Day-13	
—18	302	212
—15	318	274
—13	318	274
	Total 938	760
—10	Sheep 19 slaughtered	
	Sheep 20 larval viability control Day-11 to Day-7	
—11	320	274
— 9	96	—
— 7	84	—
	Total 500	274
— 4	Sheep 20 slaughtered	
	Sheep 21 larval viability control Day-6 to Day-1	
— 6	168	—
— 5	126	—
— 4	126	—
— 3	123	—
— 2	146	—
— 1	146	—
	Total 835	—
+ 3	Sheep 21 slaughtered	

With the aid of a multiple comparison technique like the Student-Newman-Keuls test, it can be shown that the mean of the control group differs significantly from the other two but that there is no significant difference between the means of the two treated groups.

Comment

The large coefficients of variation in this experiment immediately attracted our attention. This phenomenon appears to be quite common in anthelmintic tests (e.g. see data in Pretorius, 1967; Shone & Philip, 1967). These large variations may be due to differing host-parasite reactions or to inefficient worm recoveries.

A further experiment, with more efficient anthelmintics and using larger groups of sheep, was carried out.

EXPERIMENT 2

Materials and Methods

1. Thirty-three weaned Dorper sheep born, reared and maintained worm-free were used.

2. Instead of a single Day 0 control, three animals (Sheep 19, 20 and 21) were used as indicators of larval viability (larval viability controls). The number of infective larvae of *O. columbianum* and *Dictyocaulus filaria* (Rudolphi, 1809) dosed and the time of slaughter are shown in Table 3.

3. The remaining 30 sheep were dosed with the number of infective larvae of *O. columbianum* and *D. filaria* and the time of treatment and slaughter are shown in Table 4.

4. Each specimen was re-examined by a worker other than the one who had recovered the worms from the first examination, otherwise all procedures were identical to those described in Experiment 1.

Results

1. *Larval viability controls:* Examination of the mesenteric lymph nodes and lungs of Sheep 19 yielded only seven *D. filaria* worms out of a total of 760 larvae dosed. Since the vast majority of the larvae in the culture was not viable the infestation of Sheep 20, and of the balance of the surviving sheep in the experiment, with this parasite was discontinued on Day 11 and they were not examined *post mortem* for this species.

TABLE 4. — *Experiment* 2. *Experimental design continued*

Day	No. of infective larvae dosed to each sheep	
	Oesophagostomum columbianum	*Dictyocaulus filaria*
—18	151	106
—15	159	137
—13	159	137
—11	160	137
— 9	48	—
— 7	44	—
— 6	44	—
— 5	44	—
— 4	44	—
— 3	41	—
— 2	48	—
— 1	48	—
Total	990	517
0	Sheep 32 to 41 inclusive treated at 15 mg/kg with dl-tetramisole intraruminally Sheep 42 to 51 inclusive treated at 85 mg/kg with thiabendazole intraruminally	
+ 3	Slaughtered Sheep 22, 23 & 24, Day +3 Controls Slaughtered Sheep 32, 33 & 34, treated on Day 0 with dl-tetramisole Slaughtered Sheep 42, 43, 44 & 45, treated on Day 0 with thiabendazole	
+ 4	Slaughtered Sheep 25, 26 & 27, Day +4 Controls Slaughtered Sheep 35, 36, 37 & 38, treated on Day 0 with dl-tetramisole Slaughtered Sheep 46, 47 & 48, treated on Day 0 with thiabendazole	
+ 5	Slaughtered Sheep 28, 29, 30 & 31, Day +5 Controls Slaughtered Sheep 39, 40 & 41, treated on Day 0 with dl-tetramisole Slaughtered Sheep 49, 50 & 51, treated on Day 0 with thiabendazole	

Sheep 19, 20 and 21 yielded 14, 41 and 72 *O. columbianum* respectively, distributed between the third and fourth stage. The real reasons for these poor recoveries are not known, although some specimens were accidentally discarded. Such accidents were avoided subsequently in the main part of this experiment.

2. *Anthelmintic test:* The results are summarized in Table 5. Since the number of worms recovered exceeded the number of infective larvae thought to have been dosed it is obvious that more were dosed than were shown in Table 4.

Since more than 990 larvae were dosed, it is not surprising that there was a great range in the worm burdens of the controls, i.e. from 193 to 1,280 with a mean of 801.4 and a standard deviation (s.d.) of 370.9. A similar range has already been noted where more than 2,000 larvae were dosed (Reinecke 1966b); more uniform worm burdens resulted when only 600 to 900 infective larvae were dosed (Reinecke, 1966a; 1967).

An F-test revealed highly significant differences among the means and a subsequent Student-Newman-Keuls test showed that all three means differ highly significantly from each other.

Comment

The anthelmintics used are known to eliminate the fifth stage of *O. columbianum*, which accounts for their absence. Although a comparison of the means showed a reduction varying from 61.9 to 71.9 per cent with tetramisole and 85.0 and 90.9 per cent with thiabendazole, both groups contained sheep whose worm burdens greatly exceeded those in some of the controls.

Third stage larvae in the various groups could not be compared with any confidence because they were present in such small numbers in most of the controls, and completely absent in Sheep 22.

Larvae in the fourth stage, however, formed the bulk of the infestations present and were reflected in the total counts. These total counts showed that five sheep in the group treated with tetramisole (Sheep 32, 33, 35, 36 and 39) and one in the thiabendazole group (Sheep 51) had worm burdens within the range of the controls. In the tetramisole group three exceeded the second lowest and two the third lowest worm burden of the controls. When these facts are taken into account the reductions on the means by 71.9 per cent for tetramisole and 90.9 per cent for thiabendazole may lead to false interpretations of the results.

The interpretations of these results by comparing the means could hardly be used for the assessment of anthelmintic efficacy. The coefficients of variation were even larger than in the previous experiment.

A third experiment was planned in which two groups of sheep were infested at two different levels. No anthelmintics were used.

TABLE 5. — *Number of* Oesophagostomum columbianum *recovered at autopsy*

Group	Sheep No.	Stage of development			Total
		L_3	L_4	5	
Controls:					
Killed on Day + 3	22	0	1,155	125	1,280
Killed on Day + 3	23	29	1,068	0	1,097
Killed on Day + 3	24	10	718	32	760
Killed on Day + 4	25	103	311	0	414
Killed on Day + 4	26	180	960	18	1,158
Killed on Day + 4	27	63	793	0	856
Killed on Day + 5	28	78	275	0	353
Killed on Day + 5	29	13	150	30	193
Killed on Day + 5	30	33	1,016	11	1,060
Killed on Day + 5	31	37	788	18	843
	Mean	54.6 s.d. 54.5	723.4 s.d. 113.3	23.4 s.d. 37.9	801.4 s.d. 370.9
Treated on Day 0 with dl-tetramisole at 15 mg/kg					
Killed on Day + 3	32	22	432	0	454
Killed on Day + 3	33	28	238	0	266
Killed on Day + 3	34	0	53	0	53
Killed on Day + 4	35	76	410	0	486
Killed on Day + 4	36	43	331	0	374
Killed on Day + 4	37	5	61	0	66
Killed on Day + 4	38	4	21	0	25
Killed on Day + 5	39	7	244	0	251
Killed on Day + 5	40	7	89	0	96
Killed on Day + 5	41	16	168	0	184
	Mean	20.8 s.d. 23.5	204.7 s.d. 150.8	0	225.5 s.d. 169.4
	Mean Reduction	61.9%	71.7%	100.0%	71.9%
Treated on Day 0 with thiabendazole at 85 mg/kg					
Killed on Day + 3	42	0	8	0	8
Killed on Day + 3	43	16	67	0	83
Killed on Day + 3	44	6	43	0	49
Killed on Day + 3	45	0	13	0	13
Killed on Day + 4	46	11	109	0	120
Killed on Day + 4	47	22	71	0	93
Killed on Day + 4	48	7	43	0	50
Killed on Day + 5	49	2	34	0	36
Killed on Day + 5	50	10	61	0	71
Killed on Day + 5	51	8	197	0	205
	Mean	8.2 s.d. 7.0	64.6 s.d. 55.1	0	72.8 s.d. 58.2
	Mean Reduction	85.0%	91.1%	100.0%	90.9%

TABLE 6. — *Experiment* 3. *Experimental design. Larval viability controls*

Day	No. of infective larvae of *N. spathiger* dosed
	Sheep 52, larval viability control, Day-14 to Day-8
—14	613
—12	636
—10	600
— 8	608
	Total 2,457
— 5	Sheep 52 slaughtered
	Sheep 53, larval viability control, Day-6 to Day-2
— 6	600
— 4	562
— 2	592
	Total 1,754
+ 1	Sheep 53 slaughtered

TABLE 7. — *Experiment* 3. *Experimental design continued*

Day	No. of infective larvae of *N. spathiger* dosed to each sheep			
	Group 1		Group 2	
—14	613			292
—12	636			318
—10	600			300
— 8	608			304
— 6	600			300
— 4	560			280
— 2	592			296
	Total	4,209		2,090
0	Slaughtered Sheep 54, Group 1			
+ 1	Slaughtered Sheep 55 to 59 inclusive, Group 1 Slaughtered Sheep 64 to 69 inclusive, Group 2			
+ 2	Slaughtered Sheep 60 to 63 inclusive, Group 1 Slaughtered Sheep 70 to 74 inclusive, Group 2			

TABLE 8. — *Number of* Nematodirus spathiger *recovered at autopsy*

Group	Sheep No.	Stage of development				
		L_3	L_4	5	Adult	Total
Group 1 Total No. of infective larvae dosed = 4,209						
Killed on Day 0	54	172	1,249	265	51	1,737
Killed on Day +1	55	250	1,434	507	82	2,273
Killed on Day +1	56	149	894	585	93	1,721
Killed on Day +1	57	267	1,041	436	90	1,834
Killed on Day +1	58	496	1,866	507	89	2,958
Killed on Day +1	59	274	1,004	517	88	1,883
Killed on Day +2	60	138	877	307	56	1,378
Killed on Day +2	61	94	235	1	0	330
Killed on Day +2	62	162	1,115	727	276	2,280
Killed on Day +2	63	60	695	524	184	1,463
	Mean	206.2 s.d. 124.4	1,041.0 s.d. 435.0	437.6 s.d. 201.7	100.9 s.d. 76.8	1,785.7 s.d. 688.3
Group 2 Total No. of infective larvae dosed = 2,090						
Killed on Day +1	64	45	495	334	65	939
Killed on Day +1	65	53	516	248	46	863
Killed on Day +1	66	170	647	186	114	1,117
Killed on Day +1	67	127	665	346	84	1,222
Killed on Day +1	68	172	466	197	4	839
Killed on Day +1	69	27	631	342	64	1,064
Killed on Day +2	70	75	712	122	19	928
Killed on Day +2	71	67	613	396	95	1,171
Killed on Day +2	72	197	579	186	40	1,002
Killed on Day +2	73	150	655	171	44	1,020
Killed on Day +2	74	123	750	300	115	1,288
	Mean	109.6 s.d. 58.7	611.7 s.d. 89.8	257.1 s.d. 90.3	62.7 s.d. 36.6	1,041.2 s.d. 146.5

EXPERIMENT 3

Materials and Methods

1. Twenty-three weaned Merino sheep born, reared and maintained worm-free were used.

2. Two larval viability controls (Sheep 52 and 53) were dosed with infective larvae of *Nematodirus spathiger* (Railliet, 1896) and slaughtered as shown in Table 6.

3. The remaining 21 sheep were divided into two groups, infested at different levels with infective larvae of *N. spathiger*. The number of larvae dosed, days of dosing and slaughter are indicated in the experimental design (Table 7).

4. After slaughter worms were recovered by the methods of Shone & Philip (1967) and Reinecke (1967) and heat-killed as described by Reinecke (1968).

5. Larval stages were identified according to the description of Kates & Turner (1955).

Results

Larval viability controls: In Sheep 52, 130 third stage and 780 fourth stage larvae were present and in Sheep 53, 146 third stage and 535 fourth stage larvae. This highly satisfactory result indicated that the infective larvae dosed throughout the period of infestation were viable. Since these larvae were harvested from a single culture, which was used for all sheep in this experiment, any large variations in the worm burdens were not due to inability of the infective larvae to develop in the host.

Group 1 (*See Table* 8): The range varied from 330 worms in Sheep 61 to 2,958 in Sheep 58, with a mean of 1785.7 and a s.d. of 688.3. As it had been shown that the larvae were fully viable this range was apparently the result of host reaction. In Sheep 61 there was obviously a host reaction to the detriment of all stages of development, especially the fifth stage, in which there was only one worm, and the adult stage, which was absent.

Group 2 (*Table* 8): The range varied from 839 (Sheep 68) to 1,288 (Sheep 74) and the mean and s.d. were 1,041.2 and 146.5 respectively. These results demonstrate that it is possible to get a reasonable coefficient of variation in a group of untreated sheep. Whether this would still be the case after treatment is doubtful.

Two procedures described in the materials and methods materially assisted in the achievement of accurate counts.

1. Worms killed with heat relax and do not bunch together in knots, as in the case when they are killed with iodine (Reinecke, 1967). This means that all the worms were free and could be counted individually.

2. Almost all the larval stages of *N. spathiger* migrate through the nylon mesh into the filtrate, in common with other species (Reinecke, 1967). In this trial, however, a mean of 8.4 per cent remained in the residue, although in two animals (Sheep 65 and 56) this figure rose to 26.4 and 30.1 per cent respectively. Although most of the worms were in the fifth stage, it is obvious that if we had neglected to carry out the laborious examination of the residue these worms would have been missed.

Comment

In the previous experiments there is evidence that the number of helminths in a group of sheep does not follow a normal distribution. This inva-

lidates the use of statistical techniques based on the normal distribution. Moreover, it appears that the distribution of the worm burdens of treated animals differs from that of untreated controls. Therefore, transformations are also impracticable.

The use of a non-parametric method, employing population percentiles, seems the safest course, rather than the use of means and other moments. The following method was therefore evolved.

A method for evaluating results

General theory

The method proposed deals with a case in which it is impossible or impracticable to measure a response more than once on the same animal, e.g. when the individual has to be killed before its response can be measured and it cannot therefore serve as its own control.

Two populations, known as the 'control' and 'treated' populations respectively, will be considered. The former is the hypothetical population of all untreated individuals while the latter is the hypothetical population of all the treated individuals. A comparison has to be made between certain distributional properties of a variable in the control population with those in the treated population. More specifically, we wish to determine by means of this comparison whether the treatment was effective or not.

Let the variable under consideration be x and the distribution function of x in the control population, be denoted by $f_c(x)$ and in the treated population by $f_t(x)$.

A useful criterion of efficacy may be stated as follows:—

A treatment t is effective if at least 100.p′ per cent of $f_t(x)$ is smaller (or greater) than $q.\theta_p$; where θ_p is the 100.p′ per cent point of $f_c(x)$ and q,p and p′ ($0 < p, p' < 1$) are arbitrarily stated constants. A treatment is regarded as ineffective if it does not measure up to this standard.

This criterion must now be investigated using the experimental results from two groups of animals, the control and the treated animals.

We thus have samples of $f_c(x)$ and $f_t(x)$ from which we must decide whether or not the treatment was effective. A method of arriving at a decision will now be proposed. Since this method is non-parametric no assumptions are necessary as regards $f_c(x)$ and $f_t(x)$ or their parameters, except a minor one which will be mentioned below.

First the lower or upper confidence limit for θ_p from the control sample must be calculated. If the criterion of efficacy specifies that the least 100.p′ per cent of $f_t(x)$ must be smaller than $q.\theta_p$ the lower limit (L_L) is calculated. [Conversely, if $f_t(x)$ must be larger than $q.\theta_p$, the upper limit (L_U) is calculated, but this does not apply to anthelmintic tests.] The lower limit, L_L, rather than a point of θ_p, is used to ensure that the minimum requirements of the criterion are being met.

Walsh (1962, p. 205) described a method using the "interpolated confidence interval" to calculate these limits. In this method it is necessary to assume that there is continuity of x in the θ_p point. The method proceeds as follows:—

Let $X[1]_c, X[2]_c, \ldots\ldots, X[n_c]_c$ arranged from the smallest value to the largest, be the sample from the control population. The probability that $X[u]_c$ is smaller than θ_p is

$$\sum_{i=u}^{n_c} \binom{n_c}{i} p^i (1-p)^{n_c - i}$$

thus: $$P[X[u]_c < \theta_p] = \sum_{i=u}^{n_c} \binom{n_c}{i} p^i (1-p)^{n_c - i} \quad \ldots (1)$$

In future this function will be referred to as b(u).

Suppose $1 - \alpha$ was chosen as the probability that $L_L < \theta_p$ or that $L_U \geq \theta_p$. (The probability that $L_U < \theta_p$ will then be α.) Now u or u′ must be determined so that $b(u) \geq 1 - \alpha \geq b(u+1)$ or $b(u') \geq \alpha \geq b(u'+1)$ respectively for the lower and upper limits. According to Walsh (1962) L_L will now be given by the equation:

$$[(K_{1-\alpha} - K_{b(u+1)})/(K_{b(u)} - K_{b(u+1)})] \times [u] + [(K_{b(u)} - K_{1-\alpha})/(K_{b(u)} - K_{b(u+1)})] \times [u+1]; \ldots (2)$$
$$P[L_L < \theta_p] \doteq 1 - \alpha$$

where K_ϕ is the $(1-\phi).100$ percentage point of the standardised normal frequency distribution; and L_U will be given by

$$[(K_\alpha - K_{b(u'+1)})/(K_{b(u')} - K_{b(u'+1)})] \times [u'] + (K_{b(u')} - K_\alpha)/(K_{b(u')} - K_{b(u'+1)})] \times [u'+1]; \ldots (3)$$
$$P[L_U \geq \theta_p] \doteq 1 - \alpha$$

Note: The desired probability, $1 - \alpha$, is only approximately satisfied by the estimates of L_L and L_U. The true value, however, is bounded between two known values which are usually at least moderately close together.

We have now calculated L_L or L_U from the control sample. The next step is to determine from the treated sample whether it can be assumed that at least 100.p′ per cent of $f_t(x)$ is smaller than $q.L_L$, or greater than $q.L_U$ whichever of the two hypotheses is of interest.

Let v be the number of observations in the treated sample smaller than $q.L_L$. Under the null hypothesis that 100.p′ per cent of $f_t(x)$ is *smaller* than or equal to $q.L_L$, the probability of obtaining v or *more* observations *smaller* than $q.L_L$ in a treated sample consisting of n_t individuals is:

$$\sum_{i=v}^{n_t} \binom{n_t}{i} (p')^i (1-p')^{n_t - i} \quad \ldots\ldots\ldots\ldots (4)$$

The null hypothesis is rejected if this probability is equal to or smaller than a specified small value, α', such as $\alpha' = 0.05$. In its place we accept the alternative hypothesis, namely that $q.L_L$ is larger than the 100.p′ percentage point of $f_t(x)$ and, therefore, that more than 100.p′ per cent of $f_t(x)$ is smaller than $q.L_L$. The treatment may, therefore, be regarded as effective.

If we wish to determine whether at least 100.p′ per cent of $f_t(x)$ is larger than $q.L_U$, the number of observations smaller than $q.L_U$ must be determined. Let v′ be this number. Under the null hypothesis, in this case that 100.(1—p′) per cent of $f_t(x)$ is smaller than or equal to $q.L_U$, the probability of getting v′ or *less* observations smaller than $q.L_U$ is:

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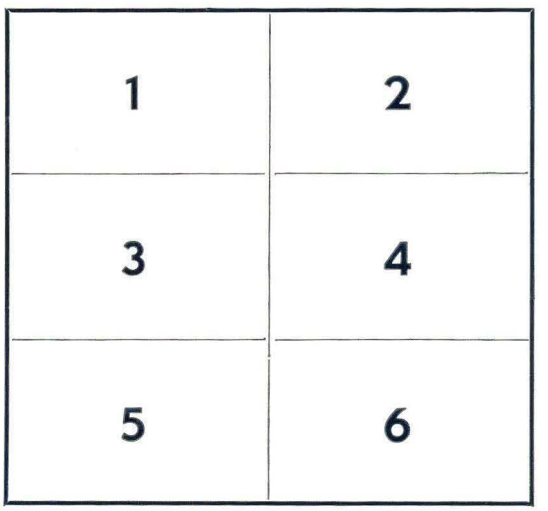
Page 189: Colour Plate 1. The sections should be numbered as follows:



Page 293: The statistical formula on top of first column should read:

$$\sum_{i=0}^{v'} \binom{n_t}{i} (1-p')^{i} (p')^{n_t - i}$$

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$$\sum_{i=0}^{v'} \binom{n_t}{i} (1-p')^i (p')^{n_t-}$$

If we reject the null hypothesis we must accept the alternative hypothesis, namely that $q.L_U$ is smaller than the $100.(1-p)'$ percentile of $f_t(x)$ and therefore that more than $100.p'$ per cent is larger than $q.L_U$.

A defect of this method is that the probabilities of incorrectly defining the treatment either as effective or ineffective are unknown. This is because the evaluation is carried out in two steps, namely the estimation of L_L or L_U, and thereafter a test to determine whether more than $100.p'$ per cent of the treated population has smaller values than L_L or larger values than L_U.

The probability of incorrectly defining the treatment as effective is rather small if a large value (0.95) is chosen for $1 - \alpha$ and a small value (0.05) for α'. However, the probability of incorrectly defining the treatment as ineffective is larger, and may be very large, particularly if n_c and n_t are small. The latter can be overcome to some extent by:—

(i) choosing a smaller value for $1 - \alpha$ (e.g. 0.90) and/or choosing a larger value for α' (e.g. 0.10).

(ii) Increasing the number of animals in both groups.

Evaluation of Anthelmintics

An anthelmintic may, for instance, be regarded as highly effective if more than 80 per cent of the treated animals have worm burdens less than two tenths of the median of the control population, i.e. the anthelmintic causes an 80 per cent reduction of the median worm burden.

These empirical constants can, of course, be modified to cover those compounds which are not highly effective.

The arbitrary constants are:—
$p = 0.5$ (the median)
$p' = 0.8$
$q = 0.2$

With the aid of the equations (1) and (2) mentioned previously, the lower limit L_L for the median may be calculated from the control group. Table 9 gives values of u and u' for a number of values of n_c (i.e. the number of controls) with $p = 0.5$ and $1 - \alpha = 0.95$ and 0.90.

TABLE 9.—*Constants for determining the positions of L_L and L_U ($p=0.5$)*

n_c	$1-\alpha=0.95$ U	U'	$1-\alpha=0.90$ U	U'
5	1	4	1	4
6	1	5	1	5
7	1	6	2	5
8	2	6	2	6
9	2	7	3	6
10	2	8	3	7
11	3	8	3	8
12	3	9	4	8
13	4	9	4	9
14	4	10	5	9

Writing the equation (2) as $K'(X\,[u]') + K''(X\,[u+1])$, the values of K' and K'' for different numbers of controls n_c with $p = 0.5$ and $1 - \alpha = 0.95$ and 0.90 are given in Table 10. Due to the symmetry of a normal distribution and of a binomial distribution with $p = 0.5$, the expressions in square brackets in the equation (3) are the same as K' and K'' in reversed order for the estimation of the upper limit of the median.

TABLE 10.—*Values for the interpolation of L_L and L_U ($p=0.5$)*

n_c	$1-\alpha=0.95$ K'	K''	$1-\alpha=0.90$ K'	K''
5	0.78	0.22	0.40	0.60
6	0.46	0.54	0.05	0.95
7	0.13	0.87	0.68	0.32
8	0.79	0.21	0.29	0.71
9	0.42	0.58	0.90	0.10
10	0.07	0.93	0.51	0.49
11	0.70	0.30	0.10	0.90
12	0.32	0.68	0.71	0.29
13	0.90	0.10	0.28	0.72
14	0.55	0.45	0.89	0.11

The number of treated animals with worm burdens smaller than the lower limit L_L of the median must now be determined.

This number will be denoted by v and if $p' = 0.8$ the equation (4) is used to calculate the probability of obtaining v or more observations smaller than L_L. Table 11 gives the minimum value of v that must be attained before the probability calculated by (4) will be smaller than or equal to 0.05, where $p' = 0.8$ and for different numbers (n_t) of treated animals.

TABLE 11.—*Minimum values of v*

n_t	14	15	16	17	18	19	20	21	22	23
v	14	15	16	17	18	19	20	21	21	22

Comment

Referring back to the first equations (1), il there are less than five animals in the controf group, b(1) will be smaller than 0.95 and b(5) will be larger than 0.05. The lower limit of the median L_L lies under X [1] and the upper limit L_U above X [5]. It is thus impossible to obtain interpolated values for L_L and L_U with $\alpha \leq 0.05$ and $p = 0.5$. Therefore a minimum of five animals is essential in the control group.

In a treated group with 13 or less animals and $p' = 0.8$ the equation (4) cannot be smaller than 0.05. A minimum of 14 animals must therefore be treated before this level can be obtained.

Table 12 gives the minimum number of treated animals necessary to obtain significance at the 0.05 and 0.10 levels for different values of p'.

It must be stressed that the minimum numbers referred to above will of necessity result in tests with low power and hence are not recommended.

TABLE 12.—*The minimum number of animals required to be treated for significance at two levels*

p′	$\alpha'=0.05$	$\alpha'=0.10$
0.5	5	4
0.6	6	5
0.7	9	7
0.8	14	11

It was mentioned in the previous section that there must be continuity of the variable x in the θ_p point. The number of parasites per animal is the variable in the evaluation of anthelmintics and this variable is not continuous. However, it usually covers a wide range of the population, from zero to several thousands, and the discontinuities are either small or very small. This method should therefore give good approximations for the estimation of L_L and L_U.

In order to demonstrate this method Experiments 4 and 5 were set up.

TABLE 13.—*Experiment 4. Experimental design*

Day	Number of infective larvae of *O. columbianum* dosed to each sheep
−6	 122
−5	 114
−4	 110
−3	 104
−2	 105
−1	 105
	Total 660
0	Killed Sheep 75, Day 0 control. Treated Sheep 82 to 95 inclusive with thiabendazole at 85 mg/kg intraruminally
+7	Killed Sheep 76 to 81 inclusive, Day +7 controls
+8	Killed Sheep 82 to 88 inclusive, treated on Day 0
+9	Killed Sheep 89 to 95 inclusive, treated on Day 0

EXPERIMENT 4

Materials and Methods

1. Twenty-one weaned Dorper lambs were transferred from pens with earth floors to pens with concrete floors which were maintained free from worms. They were weighed and treated on Day −31 with dl— tetramisole at 30 mg/kg *per os*. On Day −21 all faecal examinations for worm egg counts were negative.

TABLE 14.—*Experiment 4. No. of* Oesophagostomum columbianum *recovered at autopsy*

Group	Sheep No.	Stage of development		Total
		L_3	L_4	
Controls Killed on Day 0	75	187	2	189
Killed on Day +7	76	108	191	299
Killed on Day +7	77	82	269	351
Killed on Day +7	78	67	343	410
Killed on Day +7	79	75	150	225
Killed on Day +7	80	76	138	214
Killed on Day +7	81	71	197	268
	Mean	79.8	214.7	294.5
	s.d.	14.7	24.6	75.6
Treated on Day 0 with thiabendazole at 85 mg/kg				
Killed on Day +8	82	43	44	87
Killed on Day +8	83	18	26	44
Killed on Day +8	84	62	86	148
Killed on Day +8	85	35	11	46
Killed on Day +8	86	18	49	67
Killed on Day +8	87	38	76	114
Killed on Day +8	88	73	53	126
Killed on Day +9	89	18	17	35
Killed on Day +9	90	36	22	58
Killed on Day +9	91	47	20	67
Killed on Day +9	92	60	46	106
Killed on Day +9	93	29	14	43
Killed on Day +9	94	18	28	46
Killed on Day +9	95	59	25	84
	Mean	39.6	36.9	76.5
	s.d.	18.6	23.1	35.4

2. They were weighed again and split into two groups, one containing seven sheep to be used as controls and the other 14 sheep to be treated. They were divided in such a way that the average weight of the sheep in the control group and that of the treated group was the same.

3. Details of the infestation, treatment and times of slaughter of these animals are summarized in the experimental design (Table 13).

4. After slaughter worms were recovered similarly to those in the first two experiments. In addition total counts were made on the filtrates and digests and the worms were removed and identified. Another worker checked the filtrates and digests for any worms which had been missed. A one tenth aliquot of the residues was examined microscopically. If positive, the balance of the residue was also examined microscopically.

5. Worms in the third moult were classified as third stage larvae.

Results

These are summarized in Table 14.

The Day 0 control (Sheep 75) showed that the larvae were viable and almost entirely in the third stage. With the exception of one infective larva of *O. columbianum* in the ingesta, all the worms were recovered from the gut wall. The data from this animal are not included in the analysis of the results because it was slaughtered a week before the other controls.

The number of worms recovered from the six controls slaughtered on Day + 7 varied from 214 to 410. In one animal only (Sheep 81) was a single fourth stage larva recovered from the one tenth aliquot of the residue and the balance of the aliquot yielded a single fourth stage larva of *O. columbianum*. Aliquots from the residues of the other 20 sheep were all negative. This showed that almost all the larvae of *O. columbianum* migrated into the filtrate in contradistinction to *N. spathiger*, where a small percentage was consistently recovered from the residue.

Interpretation of Results

The first step is to estimate the lower limit L_L of the controls. There were six controls in Experiment 4 (Table 13). The constants for calculating the position of L_L are given in Table 9:

	95% Confidence	90% Confidence
n_c	u	u
6	1	1

L_L lies between the lowest worm burden (214 in Sheep 80) and second lowest worm burden (225 in Sheep 79) for both confidence limits.

In Table 10 the values for interpolation of L_L are:

	95% Confidence		90% Confidence	
n_c	K′	K″	K′	K″
6	0.46	0.54	0.05	0.95

$L_L = 214\ (0.46) + 225\ (0.54)$ or $214\ (0.05) + 225\ (0.95)$
$= 219.94$ i.e. 220 or 224.45 i.e. 224

Thus the lower limit of the median is so estimated that it can be stated with 95 or 90 per cent certainty that the median lies above it.

The next step is to estimate the figure for 80 per cent reduction in the worm burdens. The treated sheep must have less than two tenths of the lower limit of the median; hence less than

$L_L.(0.2) = 220\ (0.2)$
$= 44$ for 95% confidence
and $L_L.(0.2) = 224\ (0.2)$
$= 44.8$ i.e. 45 for 90% confidence.

If $p' = 0.8$, both at the 0.05 and 0.10 significance levels, all 14 animals treated must have worm burdens lower than 44 worms.

As only three out of the 14 treated sheep have worm burdens which meet this requirement, it cannot be stated that more than 80 per cent of the treated population will have worm burdens lower than L_L. (0.20).

If, for instance, q was chosen as 0.40 and p′ as 0.60, $q.L_L$ would have been 88 at the 95 per cent and 90 at the 90 per cent confidence limit. In both cases four of the sheep do not meet these requirements and the application of formula (4) reveals that neither at the 0.05 nor at the 0.10 significance levels can it be stated that more than 60 per cent of the treated population will have worm burdens lower than L_L. (0.40).

With p′ and q both equal to 0.50 we have $q.L_L = 110$ and 112 respectively for 95 and 90 per cent confidence. Three sheep have higher burdens than either of these figures and with the aid of (4) the probability of obtaining this or a more extreme result was found to be 0.029. Hence it may be concluded that more than 50 per cent of the treated population will have less worms than $L_L.(0.50)$.

Another experiment was carried out to demonstrate the use of this method.

TABLE 15.—*Experiment 5. Experimental design*

Day	No. of infective larvae dosed to each sheep	
	O. columbianum	*Ostertagia* spp.
—18	125	
—16	108	
—14	113	
—12	96	270
—11	—	295
—10	108	300
—9	—	270
—8	108	285
—7	—	246
—6	—	204
	Killed Sheep 96: larval viability control	
—5	—	230
—4	—	240
	Total 658	2,340
0	Killed Sheep 97 to 102 inclusive: Day 0 Controls Treated Sheep 103 to 117 inclusive with laevorotatory tetramisole at 7.5 mg/kg intraruminally	
+6	Killed Sheep 103 to 110	
+7	Killed Sheep 111 to 117	

EXPERIMENT 5

Materials and Methods

1. Twenty-two weaned Dorper lambs were placed in wormfree pens and treated with thiabendazole at 100 mg/kg on Day –25 and subsequently with rametin at 50 mg/kg on Day –21. Faecal examination after the last treatment was negative for worm eggs.

2. They were dosed with infective larvae of *O. columbianum* and a mixture of *Ostertagia circumcincta* (Stadelmann, 1894) and *Ostertagia trifurcata* Ransom, 1907. Details of the days of infestation, treatment and time of slaughter are summarized in Table 15.

3. The procedures used *post mortem* have been described in Experiment 4. The larval stages of *Ostertagia* spp. were identified according to the description of Douvres (1956).

TABLE 16. — *Experiment 5. Worms recovered at autopsy*

Group	Sheep No.	*Ostertagia* spp.					*Oesophagostomum columbianum*			
		Stage of development				Total	Stage of development			Total
		L_3	L_4	5	A		L_3	L_4	5	
Controls										
Killed on Day 0	97	51	681	263	0	995	29	136	0	165
Killed on Day 0	98	0	797	283	0	1,080	13	214	0	227
Killed on Day 0	99	3	733	95	0	831	11	154	0	165
Killed on Day 0	100	0	652	165	5	822	23	118	0	141
Killed on Day 0	101	20	526	163	0	709	11	107	0	118
Killed on Day 0	102	0	908	202	0	1,110	23	124	0	147
	Mean	12.3	716.2	195.2	0.83	924.5	18.3	142.2	0	160.5
	s.d.	21.0	119.1	69.8	2.04	160.8	7.7	38.7	0	37.0
Treated on Day 0 with laevo-rotatory tetramisole at 7.5 mg/kg										
Killed on Day +6	103	0	219	245	136	600	0	12	0	12
Killed on Day +6	104	0	182	127	66	375	0	1	0	1
Killed on Day +6	105	0	220	293	78	591	0	18	0	18
Killed on Day +6	106	0	235	129	22	386	0	6	0	6
Killed on Day +6	107	0	239	253	57	549	1	25	0	26
Killed on Day +6	108	0	385	197	158	740	0	43	0	43
Killed on Day +6	109	0	208	306	43	557	0	10	1	11
Killed on Day +6	110	0	233	210	44	487	3	21	0	24
Killed on Day +7	111	0	42	146	104	292	0	16	1	17
Killed on Day +7	112	0	10	116	101	227	0	37	39	76
Killed on Day +7	113	0	323	311	184	818	1	23	2	26
Killed on Day +7	114	0	161	237	261	659	0	67	20	87
Killed on Day +7	115	0	169	320	115	604	0	16	10	26
Killed on Day +7	116	0	71	114	74	259	1	33	2	36
Killed on Day +7	117	0	66	216	104	386	1	40	9	50
	Mean	—	184.2	214.7	103.1	502.0	0.47	24.5	5.6	30.6
	s.d.	—	103.1	74.5	62.3	176.6	0.08	17.1	10.8	24.6

Interpretation of results

The viability of the initial larval doses was confirmed by finding larval stages of both genera in Sheep 96.

The numbers of worms recovered from the other sheep are summarized in Table 16. The worms recovered from the controls indicated that fourth stage larvae of both genera were predominant on the day of treatment.

The lower limits of the median can be estimated from Tables 9, and 10. In the controls they are:—

Probability	*Ostertagia* spp.	*O. columbianum*
0.95	777.15	130.65
0.90	816.35	139.85

Reductions on the lower limit at the two limits of probability in the treated sheep are:—

Probability	Reduction	*Ostertagia* spp.	*O. columbianum*
0.05	80%	155.430	26.130
0.05	60%	310.860	52.260
0.05	50%	388.750	65.325
0.10	80%	163.270	27.970
0.10	60%	326.540	55.940
0.10	50%	408.175	69.925

The reduction in the mean of the total worm burdens was 45.7 per cent for *Ostertagia* spp. and 80.93 per cent for *O. columbianum*. It is apparent that l-tetramisole is not effective against 4-day to 12-day old *Ostertagia* spp. However, against 8-day to 18-day old *O. columbianum* the compound is more successful. In 13 out of 15 treated sheep there are less than the 52.3 and 55.9 worms required for the 60% : 60% category at both probability intervals by the non-parametric method.

SUMMARY

1. A non-parametric statistical method is described to interpret the results of controlled anthelmintic tests.

2. Tables are included which indicate the constants for determining the positions of the limits of the median, the values for interpolation of these limits and the minimum number of animals required to be treated for significance at two levels.

3. The effect of the anthelmintic can be simultaneously analysed in two ways:—

(*a*) The percentage reduction of worm burdens and
(*b*) the percentage of the treated flock that is affected by the anthelmintic.

References

DOUVRES, F. W., 1956. Morphogenesis of the parasitic stages of *Ostertagia ostertagi*, a nematode of ruminants. *J. Parasit.*, 42, 626–633.

EGERTON, J. R., OTT, W. H. & CUCKLER, A. C., 1963. Methods for evaluating anthelmintics in the laboratory and their application to field conditions. *Proc. Int. Conf. Wld Ass. vet. Parasit.*, 1, Hanover, 1963. The evaluation of anthelmintics. 46–52.

KATES, K. C. & TURNER, J. H., 1955. Observations on the life-cycle of *Nematodirus spathiger* a nematode parasite in the intestine of sheep and other ruminants. *Am. J. vet. Res.*, 16, 105–115.

PRETORIUS, J. L., 1967. The anthelmintic activity of tetramisole against gastro-intestinal worms and lung worms in sheep. *Jl S. Afr. vet. med. Ass.*, 38, 157–162.

REINECKE, R. K., 1963. Methods of testing anthelmintics in sheep. *Jl S. Afr. vet. med. Ass.*, 34, 233–246.

REINECKE, R. K., 1966a. A larval anthelmintic test. *Jl S. Afr. vet. med. Ass.*, 37, 27–31.

REINECKE, R. K., 1966b. The value of uniform worm burdens in the larval anthelmintic test. *Jl S. Afr. vet. med. Ass.*, 37, 133–142.

REINECKE, R. K., 1967. Improved methods for the recovery of parasitic nematodes at autopsy. *Onderstepoort J. vet. Res.*, 34, 547–562.

REINECKE, R. K., 1968. An anthelmintic test for larval stages of sheep nematodes. *Onderstepoort J. vet. Res.*, 35, 287–298.

REINECKE, R. K. & ANDERSON, P. J. S., 1967. Modifications to the larval anthelmintic test. *Jl S. Afr. vet. med. Ass.*, 38, 231–238.

REINECKE, R. K., SNIJDERS, A. J. & HORAK, I. G., 1962. A modification of standard procedures for evaluating the relative efficacy of anthelmintics. *Onderstepoort J. vet. Res.*, 29, 241–257.

REINECKE, R. K., HORAK, I. G. & SNIJDERS, A. J., 1963. Techniques for testing anthelmintics against immature *Oesophagostomum columbianum*. *Proc. Int. Conf. Wld Ass. Adv. vet. Parasit.*, 1, Hanover, 1963. The evaluation of anthelmintics. 167–180.

SHONE, D. K. & PHILIP, J. R., 1967. Anthelmintic and toxicity studies with tetramisole: I. Anthelmintic efficacy. *Jl S. Afr. vet. med. Ass.*, 38, 165–176.

VEGLIA, F., 1923. Preliminary notes on the life-history of *Oesophagostomum columbianum*. *Rep. Dir. vet. Res. Un. S. Afr.*, 9/10, 809–829.

WALSH, J. E., 1962. Handbook of non-parametric statistics. Investigations of randomness, moments, percentiles and distributions. Princeton, New Jersey: D. van Nostrand, Co. Ltd.