

(b) Larval ticks of *Rhipicephalus decoloratus* exposed to a temperature of -5°C .

Date.	Time kept at this Temperature.	Result.
July 5, 1908	5 hours	.. None died.
„ 10, „	10 „	.. „
„ 11, „	24 „	.. „
„ 12, „	48 „	.. Majority died.

Conclusions.—(1) Larval ticks of *Rhipicephalus decoloratus* die when exposed for thirty minutes to a temperature of -18°C .

(2) Larval ticks of *Rhipicephalus decoloratus* do not die when exposed to a temperature of -5°C . for twenty-four hours.

RÉSUMÉ.

1. A temperature of 0°C . retards the hatching of *Rhipicephalus appendiculatus* nymphae into adults.

2. A temperature of 0°C . does not interfere with the development of the parasite within the engorged nymphae.

3. A temperature of 0°C . does not kill the virus contained in engorged nymphae of *Rhipicephalus appendiculatus*.

4. Larval ticks of *Rhipicephalus decoloratus* die within thirty minutes when exposed to a temperature of -18°C .

5. Larval ticks of *Rhipicephalus decoloratus* do not die when exposed to a temperature of -18°C . for fifteen minutes.

6. Larval ticks of *Rhipicephalus decoloratus* do not die when exposed to a temperature of -5°C . for twenty-four hours.

7. The majority of larval ticks of *Rhipicephalus decoloratus* die when exposed to a temperature of -5°C . for forty-eight hours.

C.—FURTHER EXPERIMENTS WITH BILIARY FEVER IN EQUINES.

Continuing on the lines mentioned in my previous report, numbers of horses and mules were inoculated during the past year against piroplasmosis, and in the majority of cases I utilised donkey foal blood of the fourth, fifth, and sixth generations.

EXPERIMENT No. 12—(continued).

Fourth Generation.

Argentine horses injected with blood of a Transvaal donkey foal (third generation).

67. *Horse 2845.*—Injected on the 3rd July, 1907, subcutaneously with 3 c.c. blood of donkey foal 2564.

Typical reaction from the 7th day.

Piroplasms noted from the 14th day.

68. *Horse 2840.*—Injected on the 3rd July, 1907, subcutaneously with 5 c.c. blood of donkey foal 2564.

Reaction from the 8th day.

Piroplasms noted from the 11th day.

69. *Horse 2975.*—Injected on the 15th August, 1907, subcutaneously with 3 c.c. blood of donkey foal 2564.

Reaction from the 7th day.

Piroplasms noted from the 10th day.

Results.—Of three Argentine horses injected with blood of a Transvaal donkey foal (third generation) all reacted and recovered.

EXPERIMENT No. 13—(continued).

Fifth Generation.

Argentine horses injected with blood of Transvaal donkey foal immune against Piroplasma equi.

Horses 2844 and 2846 were injected on the 3rd July, 1907, subcutaneously with blood of donkey foal 2550.

14. *Horse* 2844.—Injected as above. Dose 3 c.c.
Reaction from the 6th day.
Piroplasms noted from the 15th day.
15. *Horse* 2846.—Injected as above. Dose 5 c.c.
Reaction from the 7th day.
Piroplasms noted from the 12th day.

Argentine horses 2962, 2963, and 2964 were injected on the 7th August, 1908, subcutaneously with 3 c.c. defibrinated blood of donkey foal 2550.

16. *Horse* 2962.—Injected as above.
Reaction from the 8th day.
Piroplasms noted from the 11th day.
17. *Horse* 2963.—Injected as above.
Reaction from the 10th day.
Piroplasms noted from the 18th day.
18. *Horse* 2964.—Injected as above.
Reaction from the 6th day.
Piroplasms noted from the 12th day.

Argentine horses 2965, 2966, and 2967 were injected on the 7th August, 1907, subcutaneously with 2 c.c. defibrinated blood of donkey foal 2550.

19. *Horse* 2965.—Injected as above.
Reaction from the 6th day.
Piroplasms noted from the 12th day.
20. *Horse* 2966.—Injected as above.
Reaction from the 9th day.
Piroplasms noted on the 18th and 19th days.
21. *Horse* 2967.—Injected as above.
Reaction from the 9th day.
Piroplasms noted from the 13th day.

Argentine horses 2981, 2982, 2983, and 2984 were injected on the 9th August, 1907, subcutaneously with 2 c.c. blood of Transvaal donkey foal 2550.

22. *Horse* 2981.—Injected as above.
Very slight reaction.
Piroplasms noted on the 13th day.
23. *Horse* 2982.—Injected as above.
Reaction from the 7th day.
Piroplasms noted on the 12th and 13th days.
24. *Horse* 2983.—Injected as above.
Reaction from the 10th day.
Piroplasms noted on the 12th and 13th days.
25. *Horse* 2984.—Injected as above.
Reaction from the 7th day.
Piroplasms noted on the 11th, 12th, and 13th days.

Result.—Of twelve Argentine horses injected with blood of a Transvaal donkey foal (fourth generation) all reacted and recovered.

EXPERIMENT No. 14—(continued).

Sixth Generation.

Argentine horses injected with blood of Transvaal donkey foal immune against Piroplasma equi.

Horses 2842 and 2843 were injected on the 3rd July, 1907, subcutaneously with blood of Transvaal donkey foal 2551.

4. *Horse* 2842.—Injected as above. Dose 3 c.c.
Reaction from the 7th day.
Piroplasms noted from the 12th day.
5. *Horse* 2843.—Injected as above. Dose 5 c.c.
Reaction from the 8th day.
Piroplasms noted from the 12th day.
Argentine horses 2968, 2969, and 2970 were injected on the 7th August, 1907, subcutaneously with 3 c.c. blood of Transvaal donkey foal 2551.
6. *Horse* 2968.—Injected as above.
Reaction from the 9th day.
Piroplasms noted from the 15th day.
7. *Horse* 2969.—Injected as above.
Reaction from the 12th day.
Piroplasms noted from the 13th day.
8. *Horse* 2970.—Injected as above.
Reaction from the 7th day.
Piroplasms noted from the 15th day.

Argentine horses 2971, 2972, and 2973 were injected subcutaneously with 2 c.c. defibrinated blood of donkey foal 2551 on the 7th August, 1907.

9. *Horse* 2971.—Injected as above.
Reaction from the 7th day.
Piroplasms noted from the 12th day.
10. *Horse* 2972.—Injected as above.
Reaction from the 9th day.
Piroplasms noted from the 12th day.
11. *Horse* 2973.—Injected as above.
Reaction from the 5th day.
Piroplasms noted on the 14th and 16th days.
Died from horse-sickness contracted spontaneously on the 6th September, 1907, thirty days after injection with *Piroplasma equi*.

The following Argentine horses were injected on the 9th August, 1907, with 2 c.c. blood of Transvaal donkey foal 2551:—

12. *Horse* 2985.—Injected as above.
Reaction from the 10th day.
Piroplasms present on the 16th and 17th days.
13. *Horse* 2986.—Injected as above.
Reaction from the 7th day.
Piroplasms noted on the 11th day.
14. *Horse* 2987.—Injected as above.
Reaction from the 7th day, recording 105·6 and 106 on the 14th and 18th days respectively.
Piroplasms noted from the 11th day.

Argentine Horses 2849 and 2841 were injected on the 3rd July, 1907, subcutaneously with blood of Transvaal donkey foal 2494.

15. *Horse* 2849.—Injected as above. Dose 3 c.c.
Reaction from the 7th day.
Piroplasms noted on the 15th day.
16. *Horse* 2841.—Injected as above. Dose 5 c.c.
Reaction from the 7th day.
Piroplasms noted from the 10th day.

The following Argentine horses were injected on the 7th August, 1907, with blood of Transvaal donkey foal 2494 :—

Dose 3 c.c.

17. *Horse 2974.*—Injected as above.
Reaction from the 3rd day.
Piroplasms noted on the 13th, 14th, and 15th days.
18. *Horse 2976.*—Injected as above.
Reaction on the 5th day.
Piroplasms noted on the 12th, 13th, 14th, and 15th days. Secondary reaction from 27th day; temperature reaching 106·6 on 32nd day, and death occurring on the following day from horse-sickness.
19. *Horse 2977.*—Injected as above.
Reaction from the 7th day.
Piroplasms noted from the 11th day.
Dose 2 c.c.
20. *Horse 2978.*—Injected as above.
Reaction from the 10th day.
Piroplasms noted on the 14th day.
21. *Horse 2979.*—Injected as above.
Reaction from the 10th day.
Piroplasms noted on the 15th and 16th days.
22. *Horse 2980.*—Injected as above.
Reaction from the 5th day.
Piroplasms noted on the 13th, 14th, and 15th days.

The following Argentine horses were injected on the 9th August, 1907, with 2 c.c. blood of Transvaal donkey foal 2494 immune against *Piroplasma equi* (fifth generation) :—

23. *Horse 2988.*—Injected as above.
Reaction from the 7th day.
Piroplasms present on the 11th, 12th, and 13th days.
24. *Horse 2989.*—Injected as above.
Very slight reaction from the 7th day.
Piroplasms noted on the 13th and 14th days.
25. *Horse 2990.*—Injected as above.
Reaction from the 7th day, reaching 105·4 eight days later.
Piroplasms noted from the 13th day.

Results.—Of twenty-two Argentine horses injected with blood of Transvaal donkey foals (fifth generation) all reacted and twenty recovered. The remaining two died of horse-sickness contracted spontaneously.

EXPERIMENT No. 15.

Seventh Generation.

Argentine horse injected with blood of Transvaal horse foal 2707 (sixth generation) immune against Piroplasma equi.

1. *Horse 2847.*—Injected subcutaneously with 3 c.c. blood of Transvaal horse foal on the 3rd July, 1907.
Reaction from the 7th day.
Piroplasms noted from the 8th day.

Argentine mules injected with blood of a Transvaal donkey foal immune against Piroplasma equi.

The following mules were injected on the 25th October, 1907, with 1 c.c. blood of Transvaal donkey foal 2926—immune against *Piroplasma equi* (sixth generation).

(NOTE.—All these mules had previously been immunised and tested against horse-sickness, and were exposed to natural infection at Onderstepoort, near Pretoria, two days after injection with *Piroplasma equi*.)

2. *Mule* 3009.—Injected as above.
No distinct reaction.
3. *Mule* 3012.—Injected as above.
Slight reaction.
Blood examinations negative.
4. *Mule* 3018.—Injected as above.
Slight reaction from the 7th day.
5. *Mule* 3023.—Injected as above.
Reaction from the 6th day.
Blood examinations negative.
6. *Mule* 3025.—Injected as above.
Very slight reaction.
The lesions of poikilocytosis noted on the 9th day.
7. *Mule* 3026.—Injected as above.
Slight reaction.
Microscopical examinations negative.
8. *Mule* 2893.—Injected as above.
Very slight reaction.
9. *Mule* 2894.—Injected as above.
Very slight reaction.
10. *Mule* 2887.—Injected as above.
Slight reaction from the 8th day.
11. *Mule* 2890.—Injected as above.
Indistinct reaction.
Blood examinations negative.
12. *Mule* 2895.—Injected as above.
Slight reaction from the 8th day.
Blood examinations negative.
13. *Mule* 2896.—Injected as above.
Reaction from the 7th day.
14. *Mule* 2892.—Injected as above.
Reaction from the 6th day.
15. *Mule* 2898.—Injected as above.
Reaction from the 6th day.
Blood examinations negative.
16. *Mule* 2897.—Injected as above.
Reaction from the 6th day.
Blood examinations negative.
17. *Mule* 2928.—Injected as above.
No distinct reaction.
18. *Mule* 2889.—Injected as above.
Slight reaction.
19. *Mule* 2992.—Injected as above.
Reaction from the 7th day.
Piroplasms noted five days later.
20. *Mule* 2993.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 14th day.
21. *Mule* 2994.—Injected as above.
Slight reaction.
22. *Mule* 2995.—Injected as above.
No distinct reaction.

23. *Mule* 2996.—Injected as above.
Reaction from the 6th day.
Blood examinations negative.
24. *Mule* 2997.—Injected as above.
Reaction from the 7th day.
Piroplasms noted on the 17th day.
25. *Mule* 2998.—Injected as above.
Slight reaction.
Piroplasms noted on the 18th day.
26. *Mule* 2999.—Injected as above.
Reaction from the 6th day.
Piroplasms noted five days later.
27. *Mule* 3000.—Injected as above.
Slight reaction.
28. *Mule* 3001.—Injected as above.
Slight reaction.
29. *Mule* 3002.—Injected as above.
Very slight reaction.
30. *Mule* 3003.—Injected as above.
Reaction.
31. *Mule* 2929.—Injected as above.
Slight reaction.
32. *Mule* 2930.—Injected as above.
Slight reaction from the 5th day.
33. *Mule* 2931.—Injected as above.
Slight reaction.
34. *Mule* 2932.—Injected as above.
Slight reaction.
35. *Mule* 2933.—Injected as above.
Slight reaction.
36. *Mule* 2934.—Injected as above.
Slight reaction.

The following Argentine mules were injected on the 11th November, 1907, with 1 c.c. blood of Transvaal donkey foal 2926, sixth generation (these mules had previously been immunised and tested against horse-sickness):

37. *Mule* 3005.—Injected as above.
Very slight reaction.
38. *Mule* 3007.—Injected as above.
Slight reaction.
Blood examinations negative.
39. *Mule* 3015.—Injected as above.
Slight reaction.
The lesions of poikilocytosis noted on the 8th day.
40. *Mule* 3019.—Injected as above.
Reaction from the 7th day.
41. *Mule* 3011.—Injected as above.
Reaction from the 5th day.
42. *Mule* 3016.—Injected as above.
Reaction from the 5th day.
Blood examinations negative.

43. *Mule* 3020.—Injected as above.
Slight reaction.
Piroplasms present on the 9th day
44. *Mule* 3017.—Injected as above.
Reaction from the 7th day.
Blood examinations negative.
45. *Mule* 3006.—Injected as above.
Slight reaction.
Blood examinations negative.
46. *Mule* 3014.—Injected as above.
Reaction from the 5th day.,
Blood examinations negative.
47. *Mule* 3024.—Injected as above.
Slight reaction.
48. *Mule* 3010.—Injected as above.
Slight reaction.
Blood examinations negative.
49. *Mule* 3013.—Injected as above.
Reaction from the 5th day.
Blood examinations negative.
50. *Mule* 3021.—Injected as above.
Slight reaction.
No piroplasms noted.
51. *Mule* 3022.—Injected as above.
Slight reaction.

Transvaal donkey foal injected with blood of Transvaal donkey foal immune against Piroplasma equi.

52. *Donkey foal* 3141.—Injected on the 28th November, 1907, with 5 c.c. blood of donkey foal 2926 (sixth generation).

Reaction from the 3rd day, temperature reaching 104 three days later, when the animal died from enteritis.

The following Argentine mules were injected with 1 c.c. blood of Transvaal donkey foal 2926, immune against *Piroplasma equi* (sixth generation), on the 28th November, 1907 (all mules had previously been inoculated against horse-sickness):—

53. *Mule* 3102.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 10th day.
54. *Mule* 3103.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 10th day.
55. *Mule* 3107.—Injected as above.
Slight reaction.
Piroplasms noted on the 13th day.
56. *Mule* 3112.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 12th day.
57. *Mule* 3113.—Injected as above.
Slight reaction.
Piroplasms noted on the 9th day.
58. *Mule* 3114.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 9th day.

59. *Mule* 3099.—Injected as above.
Slight reaction.
Piroplasms noted on the 7th day.
60. *Mule* 3101.—Injected as above.
Reaction from the 4th day.
No piroplasms noted.
61. *Mule* 3108.—Injected as above.
Reaction from the 5th day.
Piroplasms noted on the 9th day.
62. *Mule* 3109.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 9th day.
63. *Mule* 3106.—Injected as above.
Reaction from the 6th day.
Poikilocytosis noted on the 12th and 13th days.
64. *Mule* 3138.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 13th day.
65. *Mule* 3110.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 12th day.
66. *Mule* 3111.—Injected as above.
Reaction from the 5th day.
Piroplasms noted on the 9th day.
67. *Mule* 3137.—Injected as above.
Reaction on the 5th day.
Piroplasms noted on the 9th day.
68. *Mule* 3140.—Injected as above.
Reaction from the 4th day.
Piroplasms noted on the 9th day.

Injections on the 28th December, 1907.

69. *Mule* 3179.—Injected as above.
Reaction from the 5th day.
Piroplasms noted from the 9th day.
70. *Mule* 3180.—Injected as above.
Slight reaction.
No piroplasms noted.
71. *Mule* 3181.—Injected as above.
Slight reaction.
No piroplasms noted.
72. *Mule* 3182.—Injected as above.
Slight reaction from the 7th day.
No piroplasms noted.
73. *Mule* 3183.—Injected as above.
Reaction from the 5th day.
No piroplasms noted.
74. *Mule* 3184.—Injected as above.
Slight reaction.
No piroplasms noted.
75. *Mule* 3185.—Injected as above.
Slight reaction.
Piroplasms noted on the 8th day.

76. *Mule* 3186.—Injected as above.
Slight reaction.
No piroplasms noted.
77. *Mule* 3187.—Injected as above.
Slight reaction.
No piroplasms noted.
78. *Mule* 3188.—Injected as above.
Reaction from the 8th day.
No piroplasms noted.

Transvaal horses injected on the 14th January, 1908, with 1 c.c. blood of Transvaal donkey foal 2926 (sixth generation) immune against Piroplasma equi.

79. *Horse* 3119.—Injected as above.
No distinct reaction.
No piroplasms noted.
80. *Horse* 3121.—Injected as above.
Slight reaction.
81. *Horse* 3074.—Injected as above.
Reaction from the 6th day.
82. *Horse* 2904.—Injected as above.
Slight reaction.
83. *Horse* 3130.—Injected as above.
Slight reaction.
84. *Horse* 3065.—Injected as above.
No distinct reaction.
No piroplasms noted.
85. *Horse* 3049.—Injected as above.
No distinct reaction.

Argentine horses injected with 1 c.c. blood of a Transvaal donkey foal on the 17th January, 1908.

86. *Horse* 3248.—Injected as above.
Slight reaction.
Piroplasms noted on the 13th and 14th days.
87. *Horse* 3249.—Injected as above.
Reaction from the 10th day.
Piroplasms noted from the 13th day.
88. *Horse* 3253.—Injected as above.
Reaction from the 8th day.
Piroplasms noted from the 12th day.
89. *Horse* 3254.—Injected as above.
Reaction from the 11th day.
Piroplasms noted from the 12th day.
90. *Horse* 3255.—Injected as above.
Reaction from the 8th day.
Piroplasms noted from the 13th day.
91. *Horse* 3257.—Injected as above.
Slight reaction.
Piroplasms noted from the 13th day.
92. *Horse* 3260.—Injected as above.
Reaction from the 10th day.
Piroplasms noted from the 13th day.
93. *Horse* 3262.—Injected as above.
Slight reaction from the 11th day.
No piroplasms noted.

Injections on the 31st January, 1908

94. *Horse* 3239.—Injected as above.
No distinct reaction.
No piroplasms noted.
95. *Horse* 3243.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 12th day.
96. *Horse* 3244.—Injected as above.
Reaction from the 6th day.
Piroplasms noted from the 12th day.
97. *Horse* 3246.—Injected as above.
Reaction from the 4th day.
No piroplasms noted.
98. *Horse* 3247.—Injected as above.
Reaction from the 7th day.
Piroplasms noted on the 14th day.
99. *Horse* 3250.—Injected as above.
No distinct reaction.
No piroplasms noted.
100. *Horse* 3252.—Injected as above.
No distinct reaction.
No piroplasms noted.
101. *Horse* 3256.—Injected as above.
Reaction from the 7th day.
No piroplasms noted.
102. *Horse* 3258.—Injected as above.
Reaction from the 6th day.
No piroplasms noted.
103. *Horse* 3259.—Injected as above.
Slight reaction.
Piroplasms noted on the 14th day.
104. *Horse* 3261.—Injected as above.
Reaction from the 3rd day.
Piroplasms noted on the 11th day.
105. *Horse* 3264.—Injected as above.
Slight reaction.
Piroplasms noted on the 14th day.
106. *Horse* 3265.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 13th day.
107. *Horse* 3266.—Injected as above.
Slight reaction.
Piroplasms noted on the 14th day.
108. *Horse* 3267.—Injected as above.
Slight reaction.
No piroplasms noted.
109. *Horse* 3268.—Injected as above.
Slight reaction.
No piroplasms noted.
110. *Horse* 3269.—Injected as above.
Reaction from the 5th day.
Piroplasms noted on the 14th day.

111. *Horse 3273*.—Injected as above.
 Reaction from the 6th day.
 Piroplasms noted on the 14th day.
112. *Horse 3274*.—Injected as above.
 No reaction.
 No piropasms noted.

RESULTS OF 112 ANIMALS INJECTED WITH BLOOD OF TRANSVAAL DONKEY
 FOALS (SIXTH GENERATION).

27 Argentine horses reacted and recovered.
 76 " mules " "
 7 Transvaal horses " "
 1 " donkey foal reacted and died of enteritis.

N.B.—In the majority of cases the temperature reactions were of a slight or indistinct character.

One Transvaal horse foal injected with blood of a Transvaal horse (sixth generation) passed through a reaction and recovered.

ANALYSIS OF RESULTS.

1. Of sixty-four Argentine horses injected with blood of Transvaal donkey foals (third, fourth, fifth, and sixth generations) all reacted to piropasmosis and recovered, except two animals which died of horse-sickness contracted spontaneously.

2. Seventy-six Argentine mules injected with donkey foal blood of the sixth generation reacted and recovered.

3. Seven Transvaal horses injected with donkey foal blood of the sixth generation reacted and recovered.

4. One Transvaal donkey foal injected with donkey foal blood of the sixth generation died of enteritis.

5. One Transvaal horse foal injected with blood of a Transvaal horse reacted and recovered.

The total inoculations obtained by adding these results to those of last year from the injection of donkey foal blood is as follows:—

Seven Transvaal horses injected with donkey foal blood (sixth generation) recovered.

Of three Transvaal horse foals injected with donkey foal blood (second and fifth generations), one died of horse-sickness contracted spontaneously.

One Transvaal mule injected with blood of Transvaal donkey foal (second generation) recovered.

Of five Transvaal donkey foals injected with blood of a Transvaal donkey foal (third, fourth, and sixth generations), one died of enteritis.

Of seventy Argentine horses injected with blood of Transvaal donkey foals (second to sixth generations), two died of horse-sickness contracted spontaneously.

Of eighty Argentine mules injected with blood of Transvaal donkey foals (fourth to sixth generations), none died.

Of ten Argentine donkeys injected with blood of Transvaal donkey foals (second and fourth generations), none died.

CONCLUSIONS.

Argentine and Transvaal horses and mules can safely be inoculated against piropasmosis by using donkey foal blood of the fourth generation and upwards.