

# Identifying *Anaplasma phagocytophilum* in the ticks of Dorset

Rachel Baylis, Joselyn Savill, Dr J Rowe, Mr S Lewis, Mrs J Wardlaw (GENESIS Lab at The Thomas Hardy School) and Dr N King (University of Exeter).

## Overview

This project investigates *Anaplasma* in ticks using the Polymerase Chain Reaction (PCR). Three hundred ticks have been collected from animals all over Dorset by volunteers. Gel electrophoresis and PCR were used to test tick DNA extract for the bacterium for *Anaplasma*. We analysed the DNA from 32 ticks.

## Aims

Through the extraction and analysis of DNA from ticks collected in Dorset we aim to:

- Identify the proportion of ticks that contain *Anaplasma* in Dorset.
- Determine if *Anaplasma* is present in Dorset .

## The bigger picture

*Anaplasma phagocytophilum* (Fig.1) is a bacterium that causes anaplasmosis in sheep and cattle, also known as tick-borne fever and pasture fever. It also causes the zoonotic disease human granulocytic anaplasmosis.

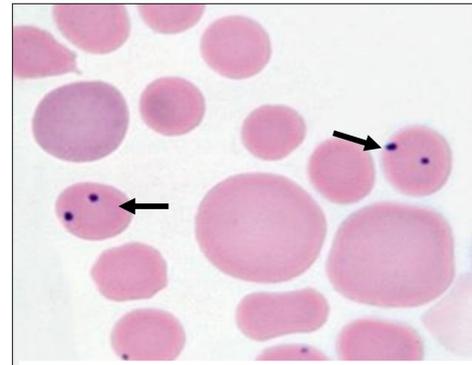


Fig. 1. Red Blood Cells from infected sample.

## Method

1. Ticks were photographed (Fig. 2) and their size, gender and location of collection were recorded.
2. Tick DNA was extracted by adding tick head and lysis buffer to a reaction tube and heated to 90° C.
3. DNA mixture, *Anaplasma* specific primers and GoTAQ were added to distilled water in an Eppendorf tube.
4. 35 cycles of PCR were used to amplify the DNA. Isolation of DNA was confirmed using primers for tick DNA.
5. DNA ladder, Positive Control and negative controls and tick DNA after PCR were pipetted into wells of agarose gel in an electrophoresis chamber.
6. Electrophoresis at 150V to separate DNA fragments into bands was carried out.
7. DNA was visualized under blue light to compare bands of DNA to the *Anaplasma* and control.

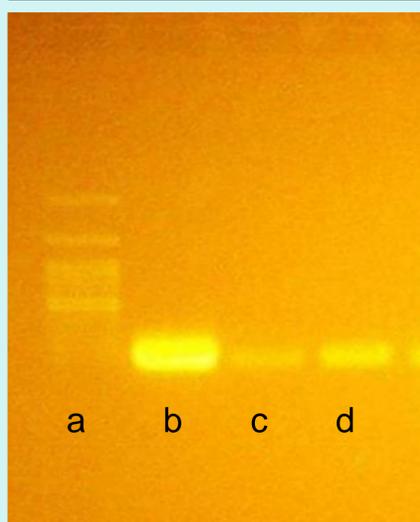


Fig. 2. Tick photographed at Thomas Hardy School.

## Results

The results for *Anaplasma* were as follows.

Positive	Unsure	Negative
5	21	6



**Fig. 3.** An electrophoresis gel visualized under blue light

- a) Ladder
- b) Positive control
- c) Negative sample
- d) Positive sample

## Conclusion

The results varied through the course of our research. Sometimes the same extract gave positive and negative results. This reduced the reliability as we could not be confident about the distribution of *Anaplasma*. The occurrence of *Anaplasma* DNA within some of our ticks was reproducibly identified, so we can conclude that the bacteria are present in Dorset, although we cannot pinpoint exactly where.

## Evaluation

If we had the opportunity to conduct our research again, we would try to collect DNA from the gut of the ticks as this is where the bacteria resides. We would also focus on improving reliability, many samples gave positive and negative results on different days, faint bands were also sometimes seen. Reducing these artefacts would improve the confidence in our results.