Animal &
Plant Health
Agency

Seroprevalence survey for *Besnoitia besnoiti* in cattle and buffalo imported to mainland UK from Europe in 2018

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Two commercially available test kits have been evaluated by APHA for performance against known positive blood samples where *B. besnoiti* was detected by Western blot and PCR analysis. The ID.vet ID Screen Besnoitia indirect ELISA kit was found to be the best performing test and is validated for diagnostic use in bovines in the UK according to APHA guidelines. A seroprevalence study of animals imported from mainland Europe was undertaken using surplus serum samples from animals eligible for bluetongue post-import testing during March to May 2018. Samples from 221 animals originating from France, Austria, Switzerland, the Netherlands and Sweden were tested using the ID.vet ELISA, all with negative results.

Introduction

Besnoitia besnoiti is an apicomplexan parasite closely related to *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp. It has been described in Africa, the Middle East and Southern Europe for over a century but since the 1990s it has been observed to be spreading within Portugal, Spain and France as well as making incursions into other European countries, most notably recently the Republic of Ireland (Ryan et al. 2016) and Switzerland (Basso et al. 2013).

It is suspected that the parasite has a similar lifecycle to other cyst-forming coccidia, with bovids as the intermediate host and a carnivore as the definitive host. However, various studies have so far failed to confirm the definitive host and the only experimentally confirmed methods of transmission among cattle are mechanical via blood sucking insects (*Tabanus* spp. and *Stomoxys calcitrans*) and iatrogenically through reusing hypodermic needles on multiple animals (Bigalke 1967 & 1968). Sheep have been occasionally found to be infected although seroprevalence studies carried out in southern Europe have failed to identify this on a significant scale and they are not thought to play a part in the epidemiology (Gutierrez-Exposito et al 2017).

It is estimated that less than one third of infected animals develop clinical signs (Fernandez-Garcia et al, 2010 and Jacquiet et al., 2010); the remaining two thirds become subclinical carriers which are considered to be epidemiologically significant and may pose a particular risk of introducing the parasite to previously unaffected areas through livestock movements. The parasite may affect cattle of all ages and breeds (although it seems to be more common in beef cattle) and can lead to substantial production losses. There is currently no effective treatment available. The infectious process has acute and chronic stages. Clinical signs in the acute phase include pyrexia, anorexia, nasal and ocular discharges, tachycardia, tachypnoea, subcutaneous oedema and orchitis. The chronic phase of infection (Fig 1) is characterised by obvious skin changes with hyperkeratosis and alopecia. Thick walled tissue cysts are visible in the scleral conjunctiva and genital mucosa. Economic losses occur due to reduced meat and milk production, hide damage, bull infertility and death/euthanasia.

Fig 1: photographs illustrating chronic Besnoitiosis (courtesy of Dr H. Cortes).

Materials and methods

Animals

221 samples were examined in total from 23 import consignments during March to May 2018: 197 domestic cattle and 24 buffalo. Samples were obtained from animals subjected to routine statutory post-import bluetongue testing and residual serum was subjected to testing with the ID Screen Besnoitia indirect ELISA kit (ID.Vet). Due to the EU import regulations only requiring the immediate premises of departure to be listed on the health certificates, detailed information on the location of the farms within the countries of origin was not available.

Serological analysis

This commercially available bovine test kit is produced by Innovative Diagnostics, Grabels, France and is an indirect ELISA method with positive and negative controls using a *Besnoitia besnoiti* purified antigen extract. Results with an S/P% value of less than or equal to 25 are negative, those with an S/P% of greater than or equal to 30 are positive, and those between 25 and 30 are doubtful. The validation for bovines was carried out by APHA Weybridge in 2017 using 50 serum samples from western blot negative animals and 51 from western blot positive animals received from the Ecole Nationale Veterinaire, Toulouse, France. The validation gave a test sensitivity of 96.23% and a specificity of 100% and the test was approved for the sero-diagnosis of *Besnoitia besnoiti* in the UK (in addition to histopathology of any suspect skin lesions). It is available as part of APHA's portfolio of bovine diagnostic tests accessible to private veterinary surgeons. The ELISA test has not been validated in the UK for use in other species.

Results

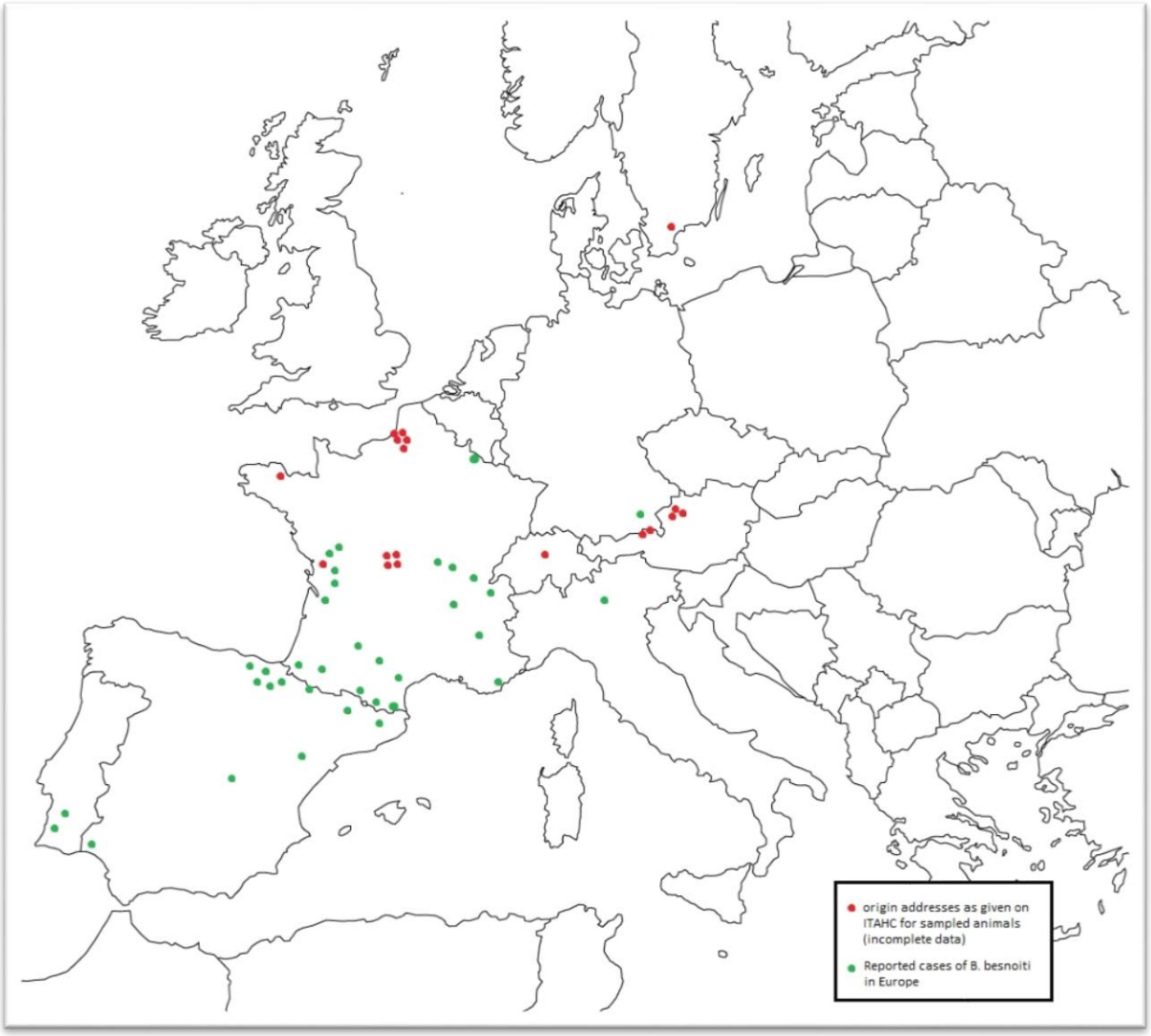
All sera tested in this study returned negative results (Table 1). The highest S/P% was 15.81 and the lowest was -1.29.

Table 1: Table summarising the ELISA results

Species	Number of animals	Country of origin	Max S/P%	Min S/P%
Cattle	11	Netherlands	2.73	0.6
Cattle	3	Austria	2.95	0.82
Cattle	1	Switzerland	2.73	N/A
Cattle	19	France	3.66	1.03
Cattle	34	France	4.21	-0.27
Cattle	1	Austria	2.62	N/A
Cattle	2	France	1.93	0.91
Cattle	15	France	7.60	2.59
Cattle	30	Austria	4.80	-1.29
Cattle	5	France	1.71	0.17
Cattle	2	France	4.44	0.46
Cattle	2	Austria	2.05	0.80
Cattle	33	France	15.81	-0.46
Cattle	2	France	4.21	1.36
Cattle	2	France	3.67	2.99
Cattle	1	France	3.13	N/A
Cattle	1	France	2.05	N/A
Cattle	5	France	15.64	1.22
Cattle	4	France	6.00	0.49
Buffalo	24	France	1.82	-1.05
Cattle	20	Sweden	7.07	2.88
Cattle	1	France	3.41	N/A
Cattle	3	France	7.81	6.07
Total sampled	221			

S/P% results	Status
<= 25	negative
>25 and <30	doubtful
>= 30	positive

Fig 2: Map illustrating assembly centre origins of sampled bovines (information not available for all consignments tested) and historical reported cases of B. besnoiti *in mainland Europe (data from EFSA Journal 2010; 8(2):1499).*



Discussion

Bovine besnoitiosis is an incurable disease associated with major economic losses and potential animal welfare issues and is currently exotic to the UK. Although the life cycle has yet to be fully determined it has already been demonstrated that biting flies (*Stomoxys* and *Tabanus*) can spread the parasite, as well as iatrogenic transmission via contaminated hypodermic needles (Bigalke 1967 & 1968), so transmission within the UK cattle population would be possible were disease to be introduced. Ryan et al (2016) reported a diagnosis of besnoitiosis in a dairy herd in the Republic of Ireland with no history of importing cattle; investigations into the source of infection are ongoing. In this case the lesions were initially thought to be due to photosensitisation, however they proved to be non-responsive to treatment. With the current trend for many UK dairy farms

to source replacement heifers from continental Europe, as well as the trade in pedigree stock, it is important to remain alert to the possibility of introduction of *B. besnoiti* via asymptomatic imported cattle.

Signs in the acute phase are non-specific and cannot easily be distinguished from a number of diseases including pyrexic viral infections such as infectious bovine rhinotracheitis and malignant catarrhal fever. Possible differential diagnoses for the chronic phase include chorioptic mange (or more rarely psoroptic or sarcoptic mange), very heavy louse infestations, photosensitivity, and severe ringworm.

The map in Fig 2 illustrates that some animals tested in this study came through assembly centres close to regions where *B. besnoiti* has been detected in the past including central France and Austria. Therefore UK veterinarians should remain alert to the possibility of this disease being introduced via imported animals. If chronic skin lesions of hyperkeratosis and alopecia are observed in a cattle herd then *B. besnoiti* should be considered as a differential diagnosis, especially if there is a history of importing animals from mainland Europe, if scleral lesions are also present, or if the skin lesions fail to respond to treatment for more common conditions.

As this would be a first diagnosis of the disease in the UK, any ELISA positives should be confirmed with a Western blot test and histopathology. Besnoitiosis is not currently notifiable by law, but it would be unfavourable for it to become established in the UK cattle population as it is incurable and has serious consequences for animal welfare and production. The validated ELISA test is available as part of APHA's standard portfolio of bovine serology testing and private veterinary surgeons are urged to consider testing any suspicious cases to aid in scanning surveillance for this potential emerging disease.

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Photographs courtesy of Dr. H Cortes, Victor Caeiro Laboratory of Parasitology, Universidade de Evora, Portugal.