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Authors: Tamieka A. Fraser, Alynn Martin, Adam Polkinghorne, Scott Carver

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Comparative diagnostics reveals PCR assays on skin scrapings is the most reliable method to detect *Sarcoptes scabiei* infestations

Tamieka A Fraser\textsuperscript{a,b,*}, Alynn Martin\textsuperscript{a}, Adam Polkinghorne\textsuperscript{b}, Scott Carver\textsuperscript{a}

\textsuperscript{a} School of Biological Sciences, University of Tasmania, Sandy Bay, 7001, TAS, Australia

\textsuperscript{b} Centre for Animal Health Innovation, University of the Sunshine Coast, 91 Sippy Downs Drive, Sippy Downs, 4556, QLD, Australia

* Corresponding author

Tamieka.Fraser@utas.edu.au

Ph: +61 3 6226 2794

Fax No: +61 3 6226 2745
Highlights

- PCR assays on skin scrapings are more sensitive at detecting mites than microscopy.
- Multiple skin scrapings from the same body segment can harbour different outcomes.
- Skin swabs are not the best alternative diagnostic method.
- Observational scoring of mange severity correlates with mite counts from microscopy on skin scrapings.
- PCRs and microscopy should be coupled for diagnosis.

Abstract

Sarcoptic mange is a globally significant parasitic disease of humans and other animals, both domestic and wild. But clinical diagnosis of *S. scabiei* infestation, using the standard skin scraping followed by microscopy technique, remains highly variable (predominantly due to false-negatives), and a major challenge for human and animal welfare. Here, we utilised a unique sample set from bare-nosed wombats (*Vombatus ursinus*) to evaluate a variety of putatively useful diagnostic approaches for *S. scabiei*. Against the standard of skin scrapings followed by microscopy, we compared observational scoring of mange severity (often employed in field studies of wildlife), PCR on skin scrapings (recently proposed as an improvement for humans and other animals), and PCR on skin swabs (proposed a non-invasive method for humans and other animals). We find that observational scoring positively correlated with counts of *S. scabiei* from skin scrapings, particularly as mange severity increases, but underdiagnoses early mange. Species-specific PCR for *S. scabiei* on skin scrapings had enhanced capacity for mite detection relative to microscopy. Finally, the non-invasive sampling method of PCR on skin swab samples had a high congruence to skin...
scraping microscopy, however prospective false negatives as a consequence to sampling is concerning. To our knowledge, this is the first study to simultaneously assess this combination of methods for S. scabiei diagnosis. We conclude that PCR on skin scrapings as an advancement on traditional microscopy, and the other techniques (observational, skin swabs and microscopy) remain useful, but harbour greater false-negatives. Outcomes are transferrable to diagnosis of S. scabiei for other host species, including humans, particularly for crusted mange and potentially ordinary mange also.

Key words: Sarcoptic mange, Sarcoptes scabiei, diagnosis, diagnostic technique, One Health

1.0 Introduction

The ectoparasitic mite Sarcoptes scabiei, is a cause of significant human and animal welfare concern, and economic burden, globally (Arlian and Morgan, 2017; McCarthy et al., 2004; Mounsey et al., 2012). This parasite causes >300 million human cases of scabies per year and has been documented to cause mange in >100 species of mammals (Engelman et al., 2013; Hicks and Elston, 2009; Tompkins et al., 2015). Pathology results from burrowing of the mite into the skin, causing irritation, inflammation, alopecia, pruritis, lesions and hyperkeratosis (Pence and Ueckermann, 2002). Two forms of disease have been described, ordinary and crusted. Ordinary scabies, the more common manifestation, results in pruritic skin lesions with mite infestations typically averaging between 5 – 15 mites per affected individual (Bhat et al., 2017). Crusted scabies, on the other hand, is a more severe and rare manifestation with thousands to millions of mites infesting an individual (Walton and Currie, 2007).

Despite its significance, clinical diagnosis of S. scabiei infestation in humans and other animals remains a challenge. Classical diagnosis involves the process of skin lesion examination and skin scraping the epidermis for visualization of the mite and/or eggs by
microscopy (Leung and Miller, 2011). This technique is commonly used by both medical practitioners for humans and veterinarians for other animals, as *S. scabiei* has a relatively distinct morphology. The level of diagnostic sensitivity using this method is well known to be variable, owing to low numbers of mites in early stages of disease and, more generally, during ordinary scabies (Skerratt, 2005; Walton and Currie, 2007). Even with crusted scabies, the ability to obtain mites from scrapings of thickened skin can be problematic (Walton and Currie, 2007).

While molecular techniques have been applied to studying the molecular epidemiology of *S. scabiei* infections in humans and animals (Arlian and Morgan, 2017; Fraser et al., 2016), their use as an ancillary diagnostic tool for detection of *S. scabiei* DNA is relatively new. A recent study used nucleic acid amplification by conventional and quantitative PCR and reported both methods to have a higher sensitivity for mite identification over microscopy in human scabies skin scrapings (Wong et al., 2015). Interestingly, analysis of swab samples by PCR collected from the crusted scabies patients (Wong et al., 2015) showed comparable results to skin scrapings, raising this as an alternative and less invasive method for collection of a scabies diagnostic specimen. Additionally, diagnosis of sarcoptic mange in non-human animals is often made by observation in field setting. Observational scoring systems are based on signs of alopecia, epidermal inflammation, hyperkeratosis and crusting, and have been used in a range of wildlife, including wolves (Almberg et al., 2012), coyotes (Samuel, 2001), chamois (Turchetto et al., 2014), and wombats (Martin et al., 2017; Simpson et al., 2016). Scoring systems assess the severity of mange disease, but are rarely evaluated against actual counts of mites.

In this study, we evaluate diagnostic methodologies for *S. scabiei* infestation. Focussing on bare-nosed wombats (*Vombatus ursinus*), we exploit a small (n=23), but rare sample set that enables comparison of PCR on skin scrapings and swabs, and observational scoring of mange
severity, to classical *S. scabiei* diagnosis based on microscopy of skin scrapings. We evaluate (a) the sensitivity and specificity of each method against microscopy of skin scrapings, and (b) the relationship between observational scoring of mange severity to mite burden. To the best of our knowledge, this is the only study to simultaneously evaluate this spectrum of *S. scabiei* diagnostic approaches.

2.0 Methods

2.1 Study system and sample collection

Within Australia, *S. scabiei* is known to infest humans, domestic and invasive mammals, and native wildlife, causing health, welfare and conservation concerns (Pence and Ueckermann, 2002; Speight et al., 2017; Tompkins et al., 2015). Bare-nosed wombats are the most impacted (*Vombatus ursinus*) wildlife species. A total of 23 bare-nosed wombats were caught, anesthetised, assessed using a mange scoring system (Simpson et al., 2016) and subjected to skin scrapings and swab collection (Figure 1) during a mange epizootic at Narawntapu National Park, Tasmania (University of Tasmania Animal Ethics No. A0014670).

The observational scoring system of mange in wombats is based on dividing each side of the wombat body into 14 different segments and scoring each segment on a 1-10 scale (Simpson et al., 2016). Wombats were classified as healthy if all body segments had a mange score of 0 – 2, and mange infested if any body segment had a score >2 (Martin et al., 2017; Simpson et al., 2016). Mange severity for individual wombats was classified by the highest body segment mange score with scores of 3 as early mange, 4 – 6 as moderate mange, 7 – 8 as severe mange and 9 – 10 as late stage mange (Martin et al., 2017).

Dry skin scrapings were taken using routine methodology and independently assessed by a veterinarian at the time of collection. Briefly, the skin of an affected area of the wombat was
pinched firmly and, at a 90º angle to the skin, a scalpel blade was used to remove skin cells prior to capillary breakage for a 1-2cm² area. Cell debris and any S. scabiei mites collected were then placed into Eppendorf tubes containing 100% ethanol for future microscopy analysis. The flank of the wombat was preferentially sampled in all cases, and additional sites were sought on 10/23 individuals opportunistically. Additionally, Catch-All™ sample collection swabs (Gene Target Solutions) were moistened with saline before application to the skin in a firm rotatory motion in a 1 cm by 2 cm area. Swabs were taken from the flank of the wombat. All skin scrapings and swabs were stored at -20ºC for future analysis.

2.2 Identification by microscopy

Identification of the S. scabiei mite from skin scrapings requires visualisation of the mite and/or eggs (Ladds, 2009). For this purpose, skin scrapings stored in ethanol were emptied into a petri dish for inspection under a dissection microscope. Mite counts were recorded for each skin scraping individually. Skin scraping samples were then returned to the Eppendorf prior to DNA extractions. At no point were samples allowed to desiccate completely or have ethanol evaporate substantially away during this process.

2.2 DNA extractions

To extract mite DNA from collected swabs, a DNA extraction was performed using a QIAamp DNA mini kit (Qiagen, Hilden, Germany), with minor modifications to the user-developed protocol (Qiagen, Hilden, Germany). Briefly, 300µL of ATL buffer was added to the swab and vortexed for 3 minutes to shake off any adhered cells and mites. The swab was removed after vortexing and this solution was heated at 56ºC overnight with the addition of 40µL of proteinase K. Purification steps were completed as per the manufacturer instructions and final DNA was eluted into 100µL of the supplied AE buffer (Qiagen, Hilden, Germany). Skin scrapings stored in 100% ethanol were spun at 17,000 x g for 5 minutes to pellet skin
debris and any mites/eggs. Ethanol was pipetted off and the pellet was air-dried at 56°C to remove any excess ethanol prior to DNA extractions. DNA extractions were completed for these skin scrapings using the above method without the 3 minutes of vigorous shaking.

2.4 *S. scabiei* cox1 and β-actin PCR

Specific *S. scabiei* primers were designed to amplify a 374 base pair (bp) fragment of the *cox1* gene for the purpose of detection of *S. scabiei* in both swab and skin scraping samples. This was designed from the alignment of new Australian marsupial (Fraser et al., 2017) and the human (Mofiz et al., 2016) *S. scabiei* mitochondrial genomes (Supplementary Figure 1). Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi#) and OligoAnalyzer 3.1 online tool (https://sg.idtdna.com/calc/analyzer) were used to analyse primers for DNA base mismatches. The total volume of each PCR assay was 25µL consisting of 1 X Amplitaq Gold 360 Master Mix (Life Technologies), 0.3 µM of forward (5’CTGGTAGAGGAAGCTGGCTG3’) and reverse (5’GTAAACTTCCGGGTGTCC3’) primers and 5µL DNA template. Cycling conditions for the thermocycler were 95°C for 10mins, 35 cycles of 95°C for 15s, 58°C for 30s and 72°C for 1 min, followed by a final extension of 72°C for 7 mins. Each reaction was visually verified by ethidium bromide on a 1.8% TBE agarose gel under UV. Sequencing PCR amplicons by Macrogen Inc. was performed on samples as well as mite DNA for validation of primer specificity. Chromatograms confirmed *S. scabiei* cox1 sequence of the correct length as indicated in Supplementary Figure 1. Detection limit of this *cox1* PCR was assessed by a serial dilution of known PCR purified product from $10^9$ to $10^0$ copies of the 374bp *cox1* fragment. By gel visualisation, we were able to detect 100 copies.

Vertebrate primers to amplify a 110bp fragment of β-actin (Shojima et al., 2013) were used to assess the quality of samples where *S. scabiei* could not be detected. Assays consisted of 2 X
Quantitect (Qiagen), 0.25µM forward and reverse primers and 5µL of DNA template. Real
time PCR conditions were 95°C for 15mins, 35 cycles of 94°C for 15s, 60°C for 30s and
72°C for 30s, and melt curve between 65°C to 95°C.

2.5 Statistical comparisons

In each case, the sensitivity, specificity and Cohen’s kappa coefficient for inter-rated
agreements of mange score, scraping PCR and swab PCR were made against the clinical
diagnostic tool of microscopy using diagnostic and agreement statistics (Mackinnon, 2000).
Additionally, to compare the relationship between mange severity score with the number of
mites obtained by skin scrapings, Spearman correlation analysis was conducted using the
statistical program, R (Team, 2013).

3.0 Results

A total of 23 bare-nosed wombats inhabiting Narawntapu National Park, Tasmania, were
sampled for this study. Observational scoring of mange severity was made on all wombats
with a total of 40 skin scrapings taken (Table 1). Of the 23 wombats, 10 had multiple (2–3)
skin scrapings taken from different body sites. For individual wombats deemed healthy or
with early mange, scraping sites were chosen that we considered likely to maximise the
probability of mite sampling. Three wombats had repeat skin scrapings taken from the same
anatomical site (Table 1). A total of 19 wombats had swab samples collected from the flank,
corresponding to skin scraping obtained.

3.1 Observational scoring shows moderate agreement to microscopy outcomes

Using the previously described mange scoring scheme for wombats (Martin et al., 2017),
13/23 (56.52%) were classified as healthy (highest body segment score ≤ 2) with seven of
these animals receiving all body segments with a mange score of 0. For those animals that
received a mange score of >2 (10/23), four were classified as having signs of early mange
(highest body segment score = 3), two with moderate mange (highest body segment score 4-6), and four at late stage mange (highest body segment score 9-10). No animal was recorded with severe mange (highest body segment score 7-8), according to the scoring scheme.

Following microscopic examination, 10/23 wombats were found to have one or more detectable mites in their skin scrapings (Table 1). For body segments that were classified as healthy (mange score ≤ 2), 6/23 (26.09%) were positive for mites microscopically. For individual body segments that were classified as mangy (body segment score >2), 7/9 (77.78%) were positive for mites. Three wombats had two or three skin scrapings taken from the same body segment (Table 1; W024, W025 and W027). W024 and W025 were classified as healthy and early, respectively, based on mange score, and an absence of mites was confirmed by the repeated skin scrapings. Wombat W027 had a mange score of 3, therefore classified as mangy, and microscopy analysis identified three, one and zero mites for this individual’s three individual skin scrapings (Table 1).

When the body segment score was compared with mite positivity and load, Spearman’s correlation analysis revealed a significant positive association between body segment mange score and mite identification ($\rho = 0.60, P < 0.001$, Fig 2). Sensitivity of observational scoring of *S. scabiei* infestation, relative to microscopy, was 57.14% (95% CI: 28.86% – 82.34%), and specificity was 88.46% (95% CI: 69.85% – 97.55%) (Table 2). Cohen’s Kappa indicated agreement between the two diagnostic tests to be moderate (47.98%, 95% CI: 19.15% – 76.80%).

**3.2 PCR on skin scrapings identifies more *S. scabiei* infested individuals than microscopy**

We applied a novel *cox1*-specific conventional PCR to the total of 40 skin scrapings collected as a part of this investigation and whose mite load was simultaneously assessed by
microscopy. In comparison to microscopy, PCR of skin scraping was 100% (95% CI: 76.84% - 100%) sensitive, with a specificity of 84.62% (95% CI: 65.13% – 95.64%). PCR negative skin scrapings were always negative by microscopy, but 4/26 samples that were negative by microscopy proved to be positive by PCR (Table 2), causing the lower specificity in this instance. The agreement between microscopy and PCR of skin scrapings was substantial (Cohan’s Kappa 79.38%, 95% CI: 60.62% – 98.14%). To validate that the four PCR positive and microscopy negative samples were not the result of non-specific amplification, sequencing was performed and confirmed *S. scabiei* PCR positivity. Haplotype sequence can be found in Supplementary Figure 1 labelled cox1 PCR sequence.

While no microscopy positive samples were negative by mite PCR, we were nevertheless concerned about an absence of amplifiable nucleic acid that could cause a ‘false-negative’ confounder in the PCR assay. As such, we separately performed an additional beta actin qPCR to test for the amplification of wombat genomic DNA in *cox1* PCR negative skin scrapings. Of the latter group of 21 negative *cox1* skin scrapings, a wombat beta actin 111 bp PCR product could be amplified for 19/21 (90.47%). The two samples that failed this control amplification (W009 and W017) produced melt curves at 80ºC, but the relative fluorescence units (RFU) were below detection levels, indicating that collection of host cells was limited but still successful.

### 3.3 PCR on swab samples may not be as effective a method for *S. scabiei* detection

We investigated the use of PCR testing of swab samples for identification of *S. scabiei* on 19/23 wombats, that were both swabbed and skin scraped at an adjacent site. Sensitivity of swab PCRs, relative to microscopy on skin scrapings was 75.00% (95% CI: 35.91% – 96.81%) and specificity 90.91% (95% CI: 58.72% – 99.77%) (Table 2). Cohan’s Kappa indicate a substantial agreement between the two diagnostic methods at 67.05% (95% CI:
33.13% – 100.98%). While the overall congruence was high, wombat beta-actin PCR testing of *cox1* PCR negative swabs only detected 2/12 (16.67%) to be positive, indicating that collection of host cells was scarce. The single false positive sample (W004) was confirmed to be positive by PCR sequencing.

### 3.4 Overall consensus identifies skin scraping PCRs are most consistent with microscopy

Nineteen wombats in the current investigation were assessed by all four sampling/diagnostic methods simultaneously (mange score, microscopic examination of skin scraping, *cox1* PCR of skin scraping and *cox1* PCR of swab sample) at the same anatomical site. Diagnostic test results for each of these is described in Table 3. Result congruence could be observed in 14/19 wombats (73.68%), comprising of 4 positive for *S. scabiei* and 10 negative. 100% agreement could be observed between microscopy and skin scraping PCR between the 19 animals (N= 8 positive, 11 negative). 84.21% congruence could be observed between microscopy/skin scraping PCR and swab PCR, with one animal (W004) being positive via swab PCR and swab PCR sequencing (and mange score of 5), but negative for microscopy and skin scraping PCR, and two wombats (W021 and W027) being negative by swab PCR but positive for the remaining methods (Table 3). These results support the above findings, particularly in regards to higher detection success of overtly healthy individuals and severe mange infestation.

### 4.0 Discussion

Despite the significance of *S. scabiei* to humans and other animals globally, clinical diagnosis of infestation (particularly false-negatives) remains a major challenge. Taking advantage of a unique sample set from bare-nosed wombats, we evaluated several techniques commonly used or recently proposed for identifying *S. scabiei* in hosts (observational scoring, PCR on
skin scrapings, and PCR on skin swabs) and compared these to the standard diagnostic of microscopy on skin scrapings. Our results show that there is a positive correlation between the presence of mites detectable by skin scrapings and observational scores. However, this technique is conservative, as the sensitivity of this approach is lower in animals with low numbers of mites. We found that *S. scabiei* specific PCR on skin scrapings had enhanced sensitivity relative to microscopic detection of the mites, and that non-invasive sampling methods via PCR on skin swab samples can also be used, but with lower sensitivity to the aforementioned methods. Overall, our results identify PCR on skin scrapings as an advancement on traditional microscopy, and highlight the more conservative nature (greater false-negatives) of other techniques tested.

Field observational diagnosis for sarcoptic mange and its severity is simple and effective for advanced mange, as indicated by alopecia and with crusted scabies due to the relatively unique signs of disease (Arlian and Morgan, 2017; Thompson et al., 2017). This method is particularly valuable as a tool for field assessment of mange, as it means animals do not need to be captured and stressed. While this methodology has been applied across several wild mammal species (e.g., Cross et al. (2016) Martin et al. (2017), the current study is the first to quantify the diagnostic relevance of observational scoring, relative to microscopic examination of skin scrapings. This analysis revealed a positive correlation between mange severity and mite load. As anticipated, the sensitivity of observational scoring was low, relative to microscopy, owing to non-detection of early stage infestation, when mite numbers are low. In a recent review, Arlian and Morgan (2017) noted that *S. scabiei* mites appear to have immune suppressing mechanisms during early stages of infestation. Even when symptoms do appear, it may be some time (weeks) before observational diagnoses can be reliably made. Accordingly, scoring of mange in field settings should always be considered
an underestimate of true prevalence (indeed most studies do), which our study suggests may be by >40% underestimation.

Our study adds to a growing body of research suggesting PCR on skin scrapings is a valuable tool for improved detection of *S. scabiei* (Alasaad et al., 2009; Bezold et al., 2001; Fukuyama et al., 2010; Wong et al., 2015). We found PCR detection of mites when they were present was largely congruent with microscopy, and detected mites in cases where microscopy did not. Interestingly, our results also provide some preliminary insight on how a single skin scraping (1-2cm² area) may be insufficient for an accurate diagnosis of low level mite infestation (one wombat, W027, receiving three skin scrapings from the same body segment were positive by microscopy 2/3 times and all three times by PCR). These results suggest that *S. scabiei* PCR assays on skin scrapings may be a more sensitive technique for detection of mites than microscopy, particularly when hosts have low levels of mite infestation. It has been suggested that mite excreta and mite cellular debris are deposited into the stratum corneum to which PCR can detect and human examination will miss (Wong et al., 2015). On the basis of these results, we suggest that, wherever possible, PCR detection of *S. scabiei* should be considered in the suite of diagnostic tools available for this disease. The obvious limitation of this latter approach is the technical and logistical requirements for a nuclear acid amplification test which may not be available in the field or in a typical veterinary clinic. In the absence of such a test, there may be opportunities in the future for the development of a point-of-care nucleic acid amplification tests for rapid, specific and low cost detection of *S. scabiei* DNA with simple benchtop equipment or even in field settings (Maffert et al., 2017). This study also offered the opportunity to evaluate the use of swabs as a new non-invasive (not skin-scraping) method of sampling *S. scabiei*. A previous human study found that skin swabs from multiple areas on the body of a single crusted scabies patient were 100% successful in detecting mite DNA by PCR, but any comparison to microscopy and scraping
PCR over the treatment period was lacking (Wong et al., 2015). In the current study we were successful in detecting mite DNA in 75% of swab samples that were also microscopically positive. Our results suggest an increased risk of false-negatives associated with PCR on swabs, relative to microscopy and also PCR on skin scrapings. This is evident by beta actin assays only confirming 16.67% of the negative cox1 samples to be positive for wombat beta actin, suggesting that the uptake of host skin containing any potential mites was insufficient. Future directions surrounding diagnostic swabs should investigate cyto-brushes as they are designed to collect a large number of host cells and minimise false-negatives.

A variety of research calls for new or additional diagnostic tools for *S. scabiei* to be coupled with the standard technique of microscopy (Lower et al., 2001; Walton and Currie, 2007; Wong et al., 2015; Zheng et al., 2016). Here, we tested a selection of putatively useful diagnostic approaches for *S. scabiei*. To the best of our knowledge, this is the first study to simultaneously assess this combination of diagnostic methods. Using our modest sample size, we identified that PCR on skin scrapings provided a higher level of detection than microscopy, and is superior to swabs for mite detection. It is suggested, however, that any PCR positive result which is not supported by microscopic identification should be additionally confirmed by amplicon sequencing. Observational scoring of *S. scabiei* infestation was also useful, but highly conservative at low levels of infestation. In spite of these methods having a high level of association, we provide evidence to suggest that a single skin scraping may be insufficient for low mite infestation as multiple skin scrapings from the same body region can produce conflicting results. The results from this unique study are not limited to wombats specifically, but can be used as fundamentals for future diagnostic research for other *S. scabiei* host species, including humans.

5.0 Abbreviations
S. scabiei: *Sarcoptes scabiei*

*cox1*: cytochrome oxidase 1

### 6.0 Acknowledgments

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### Conflict of interest

None.
References


Figure captions

Figure 1. Samples collected for each wombat for difference diagnosis methods of sarcoptic mange. Three types of samples were collected for each wombat consisting of a mange score for each body segment, a skin scraping and a swab. A total of 40 skin scrapings were obtained from 23 wombats, and only 19 swabs were collected from 19 wombats.

Figure 2. Mange score and microscopy association. 40 skin scrapings from 23 wombats, with a correlation between mange score and mite counts. Line of best fit with 95% CIs included for illustrative purposes.
Fig 1

Key
- Wombats: n= 23
- Skin scrapings: n= 40 from 23 wombats
- Swabs: n= 19 from 19 wombats

Microscopy
- Presence or absence of mites/eggs

DNA extraction
- cox 1 PCR
- Presence or absence of PCR bands (374bp)
Fig 2

![Graph showing the relationship between microscopy (mite counts) and observational score (mange severity). The x-axis represents the observational score ranging from 0 to 10, with categories for Healthy, Early, Moderate, Severe, and Late stage. The y-axis represents microscopy (mite counts) ranging from 0 to 15. The graph includes a trend line and shaded area representing the distribution of data points.](image-url)
### Tables

**Table 1. Observational classification of *S. scabiei* infestation severity and skin scraping with microscopy outcomes.**

<table>
<thead>
<tr>
<th>Wombat ID</th>
<th>Mange Classification</th>
<th>Body Segment Skin Scraping (Mange Score: Mite Tally)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW02</td>
<td>Severe</td>
<td>Flank (10: 11)</td>
</tr>
<tr>
<td>W002</td>
<td>Healthy</td>
<td>Left Flank (1: 0), Right Flank (1: 2)</td>
</tr>
<tr>
<td>W003</td>
<td>Severe</td>
<td>Flank (10: 13)</td>
</tr>
<tr>
<td>W004</td>
<td>Moderate</td>
<td>Flank (5: 0), Neck (3: 2)</td>
</tr>
<tr>
<td>W005</td>
<td>Severe</td>
<td>Flank (3: 13)</td>
</tr>
<tr>
<td>W006</td>
<td>Moderate</td>
<td>Flank (3: 2), Posterior Limb (5: 0)</td>
</tr>
<tr>
<td>W008</td>
<td>Early</td>
<td>Flank (1: 8)</td>
</tr>
<tr>
<td>W009</td>
<td>Healthy</td>
<td>Flank (0: 0)</td>
</tr>
<tr>
<td>W010</td>
<td>Healthy</td>
<td>Flank (0: 0)</td>
</tr>
<tr>
<td>W013</td>
<td>Healthy</td>
<td>Flank (0: 0), Anterior Limb (1: 0)</td>
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<td>Healthy</td>
<td>Flank (0: 0), Posterior Limb (1: 0)</td>
</tr>
<tr>
<td>W015</td>
<td>Healthy</td>
<td>Anterior Limb (0: 0)</td>
</tr>
<tr>
<td>W016</td>
<td>Early</td>
<td>Flank (2: 1), Posterior Limb (1: 4), Neck (1: 7)</td>
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<td>Healthy</td>
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<td>Healthy</td>
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<tr>
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<td>Posterior Limb (1: 0)</td>
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<td>Healthy</td>
<td>Posterior Limb (1: 0), Posterior Limb (0: 0), Shoulder (0: 0)</td>
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<td>W024</td>
<td>Healthy</td>
<td>Flank (0, 0-0)</td>
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<td>W025</td>
<td>Early</td>
<td>Flank (2, 0-0-0)</td>
</tr>
<tr>
<td>W027</td>
<td>Early</td>
<td>Flank (3: 3, 1, 0)</td>
</tr>
</tbody>
</table>

Microscopy results of 40 skin scrapings taken from 23 wombats, with mange score and mite tally for a particular skin scraping segment. Individual wombat overall mange classification/status was classified as the highest body segment having a score of 3 (early mange), 4-6 (moderate mange), 7-8 (severe mange) and 9-10 (late stage). Healthy wombats were classified by having the highest body segment with a mange score of 0-2.
Table 2: Concordance between microscopy and alternative diagnostic tests for *S. scabiei* detection.

<table>
<thead>
<tr>
<th>Alternative Test</th>
<th>Microscopy</th>
<th>Total (%)</th>
<th>Kappa (95% CI)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detected</td>
<td>Not Detected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mange Score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mangy</td>
<td>8</td>
<td>3</td>
<td>11 (27.5)</td>
<td>47.98%</td>
<td>57.14%</td>
</tr>
<tr>
<td>Healthy</td>
<td>6</td>
<td>23</td>
<td>29 (72.5)</td>
<td>(19.15 - 76.80)</td>
<td>(28.86 - 82.34)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>14 (35)</td>
<td>26 (65)</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin Scraping PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>4</td>
<td>18 (35)</td>
<td>79.38%</td>
<td>100%</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>22</td>
<td>21 (65)</td>
<td>(60.62 - 98.14)</td>
<td>(76.84 - 100)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>14 (45)</td>
<td>26 (55)</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swab PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6</td>
<td>1</td>
<td>7 (36.84)</td>
<td>67.05%</td>
<td>75.00%</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>10</td>
<td>12 (63.16)</td>
<td>(33.13 - 100.98)</td>
<td>(35.91 - 96.81)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>8 (42.11)</td>
<td>11 (57.89)</td>
<td>19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Assessment of all four diagnosis methods for 19 wombats shows a general consensus between tests.

<table>
<thead>
<tr>
<th>Wombat ID</th>
<th>Microscopy mite count</th>
<th>Body Segment</th>
<th>Skin Scraping PCR</th>
<th>Swab PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW02</td>
<td>11</td>
<td>10</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>W002</td>
<td>0</td>
<td>1</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>W003</td>
<td>13</td>
<td>10</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>W004</td>
<td>0</td>
<td>5</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>W005</td>
<td>13</td>
<td>3</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>W006</td>
<td>2</td>
<td>3</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>W008</td>
<td>8</td>
<td>1</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>W009</td>
<td>0</td>
<td>0</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>W010</td>
<td>0</td>
<td>0</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>W013</td>
<td>0</td>
<td>0</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>W014</td>
<td>0</td>
<td>0</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>W016</td>
<td>1</td>
<td>2</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>W018</td>
<td>0</td>
<td>0</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>W019</td>
<td>0</td>
<td>0</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>W020</td>
<td>0</td>
<td>0</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>W021</td>
<td>7</td>
<td>2</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>W024</td>
<td>0</td>
<td>0</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>W025</td>
<td>0</td>
<td>2</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>W027</td>
<td>3</td>
<td>3</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

19 wombats had a mange score, microscopy mite count, skin scraping PCR and swab PCR results from the same body region (flank). Mange scores between 0 – 2 are classified as healthy and mange scores >3 are classified as mangy. The remaining four wombats (W015, W017, W022 and W023) were not included in this table as skin scrapings and swabs were not collected from these individuals flank.