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Identification and disruption of bacteria associated with sheep scab mites- novel means of control?

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Abstract

Psoroptes ovis mites, which cause psoroptic mange (sheep scab), were investigated to identify potential bacterial targets for endosymbiont control of sheep scab. In addition, transmission of bacteria to the sheep skin was investigated through the characterisation of bacteria present in *P. ovis* faecal trails and on the fleece environment by internal transcribed spacer (ITS) sequencing. A diverse range of bacteria was identified in addition to a potential endosymbiont candidate, *Comamonas* sp, which was detected in *P. ovis* by both ITS PCR and endosymbiont-specific PCR. Disruption of these bacteria within *P. ovis*, through the use of antibiotics, was explored; with significant reduction in mean mite survival when administered antibiotic diets compared with controls (LR_4 = 23.12, P <0.001). The antibiotic treatments also significantly affected the bacterial density (CFU/mite) within *P. ovis*, indicating that mite survival may be linked to the bacterial communities that they harbour. Although antibiotics are not suitable for practical application, these results suggest disrupting bacteria associated with *P. ovis* should be further investigated for novel control.

Keywords: *P. ovis*, scab, sheep, endosymbiont control, internal transcribed spacer (ITS)

1 Introduction

Sheep scab is an important disease of sheep which causes significant welfare concerns. It is caused by the obligate non-burrowing mite, *Psoroptes ovis* which lives its whole life on sheep. This disease is currently treated with chemicals, either by dipping or injections, but resistance has been reported to all classes except the macrocyclic lactones (Lewis, 1997). Because of the weaknesses in control of this disease, there is a need to investigate alternatives.

P. ovis mites cause extreme pruritis (itching) and development of lesions (Baker, 1999) which may be exacerbated by opportunistic bacteria (Kirkwood, 1986). These bacteria may be ingested by mites from the skin surface (Sinclair and Filan, 1989) and subsequently potential mite luminal gut bacteria are deposited on the sheep skin in guanine-rich faecal pellets (Bates, 1999b; Lewis, 1997; Mathieson, 1995) and opportunistically infect open wounds on the sheep skin as a result of irritation (Bates, 1999a, 2003; Hogg and Lehane, 2001; Mathieson and Lehane, 1996).

There are a number of hypotheses for the presence and function of bacteria associated with *P. ovis,* as several other arthropods have close relationships with internal bacteria where they serve as a food source (Zouache et al., 2009a), or as obligate endosymbionts that are necessary for physiology and successful life cycles (Brune, 2003). The negative effect on the host of removing these endosymbionts has been shown with a number of studies (Eutick et al., 1978; Fukatsu and Hosokawa, 2002; Hogg and Lehane, 1999). Endosymbionts have been detected in many arthropod species, including predatory mite (*Metaseiulus occidentalis*) (Hoy and Jeyaprakash, 2005) and poultry red mite (*Dermanyssus gallinae*) (De Luna et al., 2009). Douglas (1989) suggested the control of arthropod pests through disruption of their endosymbionts. Bacteria have previously been observed internally within *P. ovis* (Mathieson, 1995; Mathieson and Lehane, 1996) but their function is unclear.

In this study, bacteria excreted in *P. ovis* faecal trails were compared with bacteria found on healthy and scab-infected sheep fleece to elucidate transmission of these bacteria between environments. The microbial composition of sheep fleece has been carried out previously and a shift in microbial diversity/composition has been reported to occur with disease occurrence (Lyness et al., 1994; Merritt, 1980; Merritt and Watts, 1978; Tadayon et al., 1980).

Identification of bacteria from *P. ovis* mites and the sheep fleece environment was achieved through cloning and sequencing of the internal transcribed spacer (ITS) region of bacterial DNA using PCR, where products can be separated by sequence heterogeneity to provide phylogenetic differentiation (Garcia-Martinez et al., 1999) and bacterial identification (Cardinale et al., 2004; Kolbert and Persing, 1999). Individual bacterial species can then be identified from a complex community based on the ITS sequence.

There are a number of methods previously used to disrupt endosymbionts within arthropods, including heat treatment (van Opinjen and Breeuwer, 1999), lysozyme, which destroys symbiont membranes (Nogge, 1981) and antibiotics. Removal or disruption of arthropod internal bacterial communities by antibiotics has been shown to reduce survival (Koga et al 2007), fecundity (Son et al., 2008; Zhong et al., 2007) and growth (Bandi et al., 1999; Hardie and Leckstein, 2007) but without inhibiting feeding (Ben-Yosef et al., 2008).

In this study, bacteria isolated from *P. ovis* faecal trails were used to determine effective antibiotics and concentrations through antimicrobial effect in solid and liquid cultures. These antibiotics (gentamicin and tetracycline) were then administered to *P. ovis* mites in specially constructed *in vitro* chambers to measure survival and bacterial density (Colony Forming Unit/mite). The antibiotics chosen have different bacterial targets and modes of action. Gentamicin is bactericidal, targeting Gram negative bacteria by its aminoglycosides. Tetracycline, however is bacteriostatic of both Gram positive and Gram negative bacteria by inhibiting protein synthesis within the bacteria (Hahn and Sarre, 1969). This present study aims to investigate bacteria associated with *P. ovis* mites and the effect of disrupting them on the survival of mites, for the potential novel application of parasitic control.

2 Materials

2.1 *P. ovis* samples

Mite samples (male and female) were received from SRUC Disease Surveillance Centres throughout Scotland (natural infections) and Moredun Research Institute, Edinburgh (*in vivo* cultures). Mites were used immediately for faecal trails or frozen at -80˚C for bacterial identification. For ITS-PCR three *in vivo* (M1, M2, M3) and three natural (S193, S21, S22) mite samples were selected following DNA extraction for PCR clean up, transformation and sequencing. For endosymbiont-specific PCR DNA was extracted from 14 different mite samples (eight *in vivo*, six natural).

2.2 Sheep fleece samples

Fleeces from healthy sheep were received from ewes housed at SRUC Easter Bush Estate, Edinburgh and fleeces with naturally occurring sheep scab infections were received from SRUC Disease Surveillance Centres throughout Scotland, after positive diagnosis of sheep scab infection. On receipt, samples were kept at 4°C until use as previously suggested (Lyness et al., 1994). Twenty seven fleece samples (six healthy, 21 scab-infected) were used to extract DNA, from which three samples of each (healthy H24, H91, H109; scab-infected S9, S14, S23) were used for PCR clean-up, transformation and DNA sequencing.

3 Methods

3.1 *P. ovis* faecal trails

P. ovis mites received from *in vivo* culture were used to isolate bacteria from faecal trails, following the method of Mathieson (1995). Unique colonies were picked, purified and identified by ITS- PCR and sequencing as below.

3.2 DNA extraction

Mites were surface sterilised as described in Mathieson (1995) before DNA extraction. DNA was extracted from ten mites or approximately 20 mg of fleece using phenol/chloroform extraction (Fraaije et al., 1999) with an initial homogenisation with 440 µl of 2 X TENs extraction buffer added (pH 8.0; 0.8 mM Tris-base, 0.5 mM NaCl, 0.3 mM EDTA, 1 mg/ml phenanthroline, 1µl/ml mercaptoethanol, 0.02g/ml PVP) with sterile Ballotini beads (Thistle Scientific). DNA quantity and purity was measured using a ND-1000 spectrophotometer (Nanodrop).

3.2 ITS PCR

Extracted DNA was amplified using forward primer ITSF (5'-GTC GTA ACA AGG TAG CCG TA -3') and reverse primer ITSReub (5'-GCC AAG GCA TCC ACC-3') (Cardinale et al., 2004) which targets the bacterial 16S-23S internal transcribed spacer (ITS). PCR was performed in a 25 µl reaction using 10 µl sterile water, 12.5 µl master mix (Promega; 1.5 mM MgCl2, 200 µM dNTPs, 1U colourless Go Taq), 0.5 µM primers and 2 µl DNA using a GeneAmp Thermal cycler (Biometra). Cycling conditions consisted of 2.5 min at 94°C, 30 cycles of 45 s at 94°C, 1 min at 55ºC, 1 min at 72°C, followed by a final extension of 7 min at 72°C. 20ng/µl of PCR product was run on a 2% agarose gel containing GelRed (Biotium) in TBE buffer (Eurogentec) with 100bp or

1Kb^{+} ladder for size calibration (Invitrogen). The remaining PCR product was used for cloning and sequencing.

3.3 Endosymbiotic Bacteria-Specific Primers

DNA extracted from whole *P. ovis* mites was screened for the presence of four known endosymbiont bacteria (*Wolbachia, Comamonas, Cardinium, Rickettsia*) using specific primer sets (Table 1). Optimised primer concentrations were 0.2µM (*Rickettsia*), 0.25µM (*Cardinium*, *Wolbachia*) and 0.5µM (*Comamonas*) and optimised $MgCl_2$ concentrations were 2mM (*Cardinium*, *Rickettsia*) and 2.5mM (*Comamonas*, *Wolbachia*) with 200µM dNTPs, 1.25U HotStart GoTaq (Promega UK), and 2 µl of DNA making up the PCR reaction mixture. Cycling parameters for each primer pair were as detailed in the original reference. After PCR amplification, 20ng/µl of PCR products were run on 2% agarose gels with GelRed and visualised under UV light using a ChemiImager machine. The band in the gel was cut out, purified and sequenced.

3.4 Cloning of amplified DNA

Amplified PCR products from ITS-PCR and endosymbiont-specific PCR were first purified using Roche High Pure PCR product (Roche) as per manufacturer's instructions. Purified PCR products were then ligated into pGEM-T Easy Vector (Promega, UK) following manufacturer's instructions, then 3 µl were mixed with 50 µl of JM109 High Efficiency competent cells (Promega), incubated on ice for 30 min, then heat shocked cells in a 42˚C water bath for 45 secs then returned to ice for 5 min. To this, 450 µl S. O. C medium (Invitrogen) were added then incubated with shaking at 37˚C for 1 h 20 min. Transformed cells were screened for successful inserts by blue/white colony selection and checked with M13 PCR. Following successful transformation, a single bacterial colony was used to extract purified plasmid DNA and sent to DBS Genomics, Durham University for sequencing (Applied Biosystems 3730 DNA Analyser). Chromatograms were checked after receipt using Sequence Scanner v1.0 (Applied Biosystems) and primer/vector sequences were removed. Sequences were compared to published bacterial sequences using BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1990) and values of 50% query similarity or higher were used for bacterial identification.

3.5 Effect of antibiotics on internal *P. ovis* bacteria and mite survival

To investigate the effect of disrupting internal bacteria on the survival of *P. ovis*, for each treatment 100 adult mites (50 male and female) received from *in vivo* culture, were randomly allocated to five chambers constructed based on a design by Mathieson (1995). 10 µl of the treatment diet (concentrations determined previously, data not shown; 100 µg/ml gentamicin, 100 µg/ml tetracycline in lamb serum (Invitrogen)), or either control diet of lamb serum or water (no food control) was administered daily to each mite chamber. Chambers were kept in a 26±2˚C incubator in a humid environment. Survival was recorded daily, and dead mites were removed from the chambers and frozen at -20°C. In addition three mites were removed daily from the chambers, surface sterilised as Mathieson (1995) then macerated with sterile tweezers

and mixed with 100 µl of ¼ strength Ringers solution (supplemented with 0.2% peptone; Sigma). A ten-fold dilution series in Ringers solutions was prepared from this whole-mite extract and 100 µl of three appropriate dilutions which gave approximately 100 colonies per plate were plated onto nutrient agar. The plates were incubated at 27°C for 24 h before colony forming units (CFUs) were counted.

3.6 Statistics

Survival analysis of Log rank equality of curves were carried out in Genstat (v11.1) (VSN International Ltd, UK) except Probit analysis (LT50) which was calculated in MiniTab. Bacterial density values (CFU/mite) were transformed (Log10+1) then analysed using a Linear mixed model and regression equations in Genstat (v11.1).

4 Results

4.1 Bacteria associated with *P. ovis*

54 bacterially-derived sequences were obtained from PCR amplification with 16S-23S specific oligonucleotide primers. Overall five phyla were represented, Actinobacteria, Firmicutes, Bacteroidetes, Beta-, and Gamma-proteobacteria as well as some unidentified uncultured bacteria (Table 2). Bacteria isolated from the *in vivo* cultured mites contained seven opportunistic pathogens, two pathogens, three saprophytes and two unknown. The natural infection mite samples, however, comprised of five opportunistic pathogens, one pathogen, three saprophytes, one unknown and one arthropod symbiont (Table 2).

Whole mite extracts included bacterial species previously isolated from *P. ovis*, such as *Propionibacterium acnes* and *Staphylococcus chromogenes* (Hogg and Lehane, 2001). There were also several species previously unreported as being isolated from *P. ovis,* including *Acinetobacter iwoffii*, *Moraxella osloensis*, *Pseudomonas fragi*, *Vibrio alginolyticus*, *Psychrobacter sp*., *Comamonas testosteroni*, *Janthinobacteria sp*. and uncultured *Verrucomocrobia* (Table 2). *Staphylococcus xylosus* and *Vibrio alginolyticus* were isolated from all of the natural infection mite samples, indicating a common bacterial species range irrespective of geographical origin. *Comamonas testosteroni* and *Moraxella osloensis* are known symbionts of arthropods (Zouache et al., 2009a) and nematodes (Tan and Grewal, 2001) respectively. Eleven different species were isolated from both *in vivo* cultures and natural mite samples, however, there were some differences in the species composition
(Table 2). A larger number of different *Bacillus* and *Pseudomonas* species were isolated from *in vivo* cultured mite samples compared to the natural samples. Moreover, *Actinobacteria* spp. were only detected in the *in vivo* cultured samples (Table 2).

4.2 Screening of *P. ovis* using Endosymbiont-Specific Bacterial Primers

DNA was extracted from whole mite samples and screened for the presence of *Wolbachia, Comamonas, Cardinium and Rickettsia.* Only *Comomonas,* a known arthropod-endosymbiont

(Zouache et al., 2009b), was detected in a total of 6 out of 14 *P. ovis* samples (3 *in vivo* and 3 natural).

4.3 Bacteria associated with Healthy and Scab Infected Sheep Fleece

DNA was successfully extracted from all healthy and scab-infected fleece samples except scab-infected sample S23, which failed to produce any sequences, but the reason for this is unclear. In total, 18 different species, from four phyla (Actinobacteria, Firmicutes, Gamma-proteobacteria and uncultured bacteria) were detected (Table 3). There were some species that were identified from both sample types, including *Bacteroides fragilis, Staphylococcus xylosus and* other *Staphylococcus* spp*.* Overall, a higher number of different species were detected in healthy fleece. There were some species that were only detected from scab infected fleece, all of which were designated as uncultured bacteria in the BLASTn database.

4.4 *P. ovis* faecal trails

Ten bacteria excreted by *P. ovis* onto agar were selected on the basis of their differing colony morphology and identified by DNA sequencing of the ITS region and also classified by their biological characteristics (Table 4). All isolates had BLASTn maximum identity scores greater than 71% and query coverage of greater than 50% (except one of 47%; MFB1) (Table 4). Two samples isolated most closely matched 'uncultured bacteria' which have previously be shown to provide digestive function in the wood boring beetle (*Anoplophora glabripennis*) (Geib et al., 2009).

4.5 Effect of Antibiotics on Survival of *P. ovis*

Mites were administered antibiotics (100 µg/ml gentamicin, tetracycline) to observe the effect of disrupting internal bacterial communities on mite survival. Mites from all treatments were dead by day six with the first deaths seen after day one. The longest mean survival time was observed in mites fed lamb serum (6 days) (Figure 1). A difference in survival curves was seen (LR_4 = 23.12, P <0.001) between treatments. All mites in the antibiotic treatments were dead by day three in this experiment, with LT_{50} (days) values of 2.43, 1.14, 1.87, 1.89 for lamb serum, water (no food), gentamicin and tetracycline respectively.

4.6 Effect of antibiotics on *P. ovis* bacterial density

Mites were set up in *in vitro* chambers to investigate the effect of antibiotics on *P. ovis* internal bacterial density (CFU/mite). A range of bacterial densities was recorded throughout the observation period (12-86 h) (Figure 2). The controls, water and lamb serum, peaked in CFU per mite at approximately 61 h. Gentamicin, however, appeared to reduce the CFU per mite over time with differences due to antibiotic treatment ($F_{1,51}$=4.67, P=0.039) but no significant effect of time ($F_{4,51}$=2.15, P=0.099). Tetracycline, did not appear to have a negative effect on mean CFU with a trend of increasing CFU per mite observed over time.

5 Discussion

5.1 Bacteria associated with *P. ovis* and fleece environment.

P. ovis mites harbour a community of different bacteria and no single bacterial species appears to be associated with *P. ovis* and scab-infected fleece. This suggests that *P. ovis* do not have obligate symbiotic relationships with culturable bacteria in detectable quantities. A number of bacteria were isolated from *P. ovis* mites in this study, including *Corynebacterium sp.* which have not been previously isolated from *P. ovis*. *Bacillus thuringiensis* and V. *alginolyticus* were the only bacteria isolated from both *in vivo* and natural mite samples. There were some genera, such as *Staphylococcus* spp. and *Pseudomonas* spp., which although isolated from *P. ovis* mites in this study, are too widespread in the environment to act as suitable targets for the symbiont control of sheep scab. *S. marcescens* is 'the most frequently isolated' bacteria in Psoroptes mites (Mathieson and Lehane, 1996; Perrucci et al., 2005) however, *S. marcescens* was not detected in this study, which indicates the microbial community of *P. ovis* may be dynamic. There were bacteria that were isolated from *P. ovis*, infected fleece and faecal trails (*B. cereus and S. aureus*) and another that was isolated from both faecal trails and healthy fleece (*M. luteus*).

The bacteria detected have a number of characteristics and potentially important functions, that would make them suitable for growth in the midgut of mites, such as the ability to grow under both aerobic and anaerobic conditions (*P. acnes*); ability to hydrolyse native animal proteins (*Staphylococcus spp*) and haemolytic (*Acinetobacter spp*) and proteolytic activity (*Pseudomonas spp*) (Bisset, 1962). Also Hogg & Lehane (2001) noted many species identified were characterised by their ability to produce extracellular lipase, which may aid digestion within the mite.

One known arthropod endosymbiont, *Comamonas spp*, was also detected in a natural infection of *P. ovis*. Mites were then screened with endosymbiont-specific PCR and *Comamonas spp* was detected in 43% of *P. ovis* samples. Further research into the prevalence of *Comamonas spp* in global populations of *P. ovis* is needed in addition to ascertaining its importance, function and transmission within the mite and whether it is a potential target for symbiont-control.

Sheep fleece is known to naturally contain several bacterial species including Bacillus and Staphylococci species (Lyness et al., 1994; Merritt and Watts, 1978). Moreover this community is known to alter under diseased conditions (Chin and Watts, 1992) which was observed in this study. There were several bacteria detected in healthy fleece that were also detected in mite-associated environments including: *Micrococcus luteus, Tropheryma whippeli, Bacteriodes fragilis, Staphylococcus aureus and Staphylococcus xylosus.* However, it is not possible from this study to elucidate whether the bacteria were transmitted from healthy skin to mites or whether *P. ovis* acquired them via another mode of transmission.

Use of ITS-PCR is a suitable method for molecular identification of bacterial communities due to its sensitivity, ability to detect bacteria as low as 0.1% of mixture, and can reduce potential PCR bias of preferential amplification of the same templates in a mixture (Cardinale et al., 2004). However ITS sequences on Genbank (NCBI) are limited compared to more commonly used 16s

rRNA (Danovaro et al., 2006). This may be one reason why many fleece bacterial sequences were classified as uncultured.

5.3 *P. ovis* Survival & Bacterial density

Administration of antibiotics (tetracycline, gentamicin) significantly reduced survival compared to the lamb serum control and also significantly reduced bacterial density within the mites. Although *P. ovis* mites are challenging to maintain in the laboratory, the *in vitro* chambers used in this study allowed the differentiation of effects on mite survival among treatments. Moreover the mean survival times observed were comparable to previous *in vitro* culturing efforts (Mathieson, 1995; O'Brien et al., 1994; Smith et al., 1999). Only adults were used due to the challenge of maintaining mites *in vitro* therefore it was not possible to investigate the effect of disrupting internal bacteria on other *P. ovis* life history factors such as growth, development or reproduction.

The antibiotic dose (100 µg/ml) has previously been used to investigate disruption of internal bacteria in other arthropods (Ben-Yosef et al., 2008; Douglas et al., 2006; Hardie and Leckstein, 2007; Morimoto et al., 2006; Wilkinson, 1998). Prior to survival experiments the effects of different antibiotics and concentrations on growth of *P. ovis* faecal bacteria were tested on both solid and liquid cultures and they indicated that as little as 6.25µg/ml was sufficient to reduce the bacterial population (data not shown).

The survival experiment did not discriminate which bacterial species present within *P. ovis* were being affected. It only gave an indication if the antibiotics had an effect on total bacterial abundance and mean mite survival. Also both an increase or decrease in bacterial density could indicate an effect of antibiotics, either due to direct killing or to competitive exclusion (Lan et al., 2005). Future studies could target a broader range of bacteria using multiple antibiotics simultaneously. Moreover, the change in abundance of individual bacterial species could be measured using real-time PCR (Bustin et al., 2009).

Although antibiotics were used in this study to disrupt internal bacteria, they are not suitable for long term exposure to bacteria or application as a *P. ovis* disease control due to the risk of evolution of antibiotic resistance (Bonhoeffer et al., 1997). In this study, the survival of two *P. ovis* faecal bacteria (*Carnobacterium* sp. G17and Uncultured bacteria G27) were not affected by any of the antibiotics tested.

6 Conclusion

In conclusion, *P. ovis* mites harbour a community of bacteria, some of which are excreted onto the skin of sheep in faecal pellets. This study detected for the first time *Comamonas spp*, which has been shown to be an endosymbiont bacteria in other arthropod species and could be a potential target for endosymbiont control of *P. ovis* mites in the future.

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329 Reference List

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Table 1 Primer sequences used for endosymbiont PCR screening assays with estimated amplicon length (bp). Cycling profiles were as original references. All primers targeted 16S rRNA sequences except *Wolbachia* (*Wolbachia* surface protein) (Braig et al., 1998).

Endosymbiont	Primer name	Primer sequence	Expected band length	Primer reference
Cardinium	Ch-F	5' TAC TGT AAF AAT AAG CAC CGG C 3'	500 bp	(Zchori-Fein and Perlman, 2004)
	Ch-R	5' GTG GAT CAC TTA ACG CTT TCG 3'		
Comamonas	Com199F	5' CCT TGT GCT ACT AGA AGC 3'	433 bp	(Zouache et al., 2009a)
	Com614R	5' GCA GTC ACA ATG GCA GTT 3'		
Wolbachia	81F	5' TGG TCC AAT AAG TGA TGA AGA AAC 3'	500 bp	(Braig et al., 1998)
	691R	5' AAA AAT TAA ACG CTA CTC CA 3'		
Rickettsia	EHR16SD	5’ GGT ACC YAC AGA AGA AAG TCC 3'	345 bp	(Brown et al., 2001)
	EHR16SR	5’ TAG CAC TCA TCG TTT ACA GC 3'		

Table 2. **Phylogenetic affiliation of ITS sequences of bacteria from *P. ovis* mites from natural infections and *in vivo* cultures to closest matches in BLASTn database** (http://blast.ncbi.nlm.nih.gov/Blast) with maximum identity (%) and query coverage (%). Bacterial categories classified by biological characteristics: op. path., opportunistic pathogen, sap., saprophyte, path., strict pathogen, symb., symbiont.

Phylum	Closest BLASTn match (name, accession no,)	Bacterial categories	Origin	Max identity (%) (Query coverage %)
Actinobacteria	*Corynebacterium amycolatum* , AF536501.1	op. path.	*in vivo*	87 (68)
	Propionibacterium acnes, AF386068.1	op. path.	*in vivo*	88 (67)
	Tropheryma whipplei, AJ551273.1	path.	*in vivo*	100 (98)
Firmicutes	*Bacillus cereus,* EU915688.1	op. path.	*in vivo*	100 (100)
	Bacillus cereus, GQ255884.1	op. path.	*in vivo*	100 (81)
	Bacillus thuringiensis, AM292033.1	sap.	Both	98 (98)
	Staphylococcus aureus, U39769.1	op. path.	Natural	89 (72)
	Staphylococcus chromogenes , U39770.1	op. path.	Natural	98 (66)
	Staphylococcus epidermidis, AF269309.1	op. path.	Natural	77 (68)
	Staphylococcus hyicus, U90016.1	op. path.	Natural	92 (63)
	Staphylococcus xylosus, U90017.1, U39773.1	op. path.	Natural	90 (66)
	Comamonas testoreroni, EU014531.1	symb.	Natural	97 (78)
	Acinetobacter genomosp, AY601836.1	sap.	*in vivo*	79 (86)
Beta-proteobacteria	*Acinetobacter iwoffii,* AY601835.1	sap.	Natural	93(56)
Gamma-proteobacteria	*Klebsiella oxytoca,* FJ410391.1	op. path.	*in vivo*	98 (88)
	Moraxella osloensis, EU014577.1	symb.	Natural	91 (70)
	Pseudomonas sp, AY756059.1	op. path.	*in vivo*	97 (92)
	Pseudomonas putida, EU014558.1, DQ291129.1	sap.	*in vivo*	100 (74)
	Pseudomonas mendocina, L28160.1	op. path.	*in vivo*	94 (91)
	Vibrio alginolyticus, AY245212.1	path.	Both	86 (75)
	Xanthomonas sp, DQ003226.1	sap.	Natural	99 (83)
Verrucomicrobia	*Uncultured verrucomicrobia,* AM279407.1	-	Natural	92 (51)
Uncultured bacteria	Uncultured bacterium S10-2 AB198411.1 (Ikeda et al 2005)	-	*in vivo*	94 (90)
	Uncultured bacterium O1_44 FJ356325.1 (Geib et al 2009)	-	*in vivo*	84 (90)
	Uncultured bacterium TIM15-4, AB222642.1 (Ikeda et al 2006)	-	*in vivo*	92 (79)

Table 3.Phylogenetic affiliation of ITS region sequences of bacteria from healthy and scab-infected fleece (or both) to closest matches in BLASTn database (http://blast.ncbi.nlm.nih.gov/Blast) with maximum identity (%) and query coverage (%). Bacterial categories classified by biological characteristics: op. path., opportunistic pathogen, sap., saprophyte, path., strict pathogen, symb., symbiont.

Phylum	Genera	Bacterial categories	Fleece type	Max identity (%) (Query coverage %)
Actinobacteria	*Corynebacterium sp.* BX248360.1	op. path.	Healthy	88 (54)
	Micrococcus luteus AB088764.1	sap.	Healthy	80 (100)
	Nocardia beijingensis GQ853482.1	op. path.	Healthy	95 (95)
	Rathayibacter tritici AY191505.1	path.	Healthy	76 (90)
	Tropheryma whippeli, AJ551273.1	path.	Healthy	100 (74)
	Bacteroides fragilis GQ496394.1	sap.	Both	90 (80)
Firmicutes	*Bacillus sp* AB243783.1	sap.	Healthy	94 (62)
	Bacillus fusiformis AF478083.1	sap.	Healthy	94 (60)
	Staphylococcus aureus, AF478083.1	op. path.	Healthy	94 (60)
	Staphylococcus sp. AY728162.1	op. path.	Both	94 (79)
	Staphylococcus aureus U39769.1	op. path.	Scab-infected	87 (68)
	Staphylococcus xylosus U39773.1	op. path.	Both	86 (69)
Gamma-Proteo bacteria	*Pseduomonas sp DQ003234.1*	op. path.	Healthy	74 (78)
	Pseudomonas chloroaphis DQ023306.1	sap.	Healthy	100 (65)
	Pseudomonas stutzeri U65012.1	op.path	Healthy	79 (66)
Uncultured	Uncultured 70113 AY484712.1	-	Scab-infected	96 (57)
	Uncultured bacterium AB222629.1	-	Scab-infected	81 (73)
	Uncultured Pseudomonadales AB491964.1	-	Scab-infected	90 (71)
	uncultured t1010 AF422501.1	-	Healthy	70(80)

Table 4 Identification of nine *P. ovis* faecal trail bacteria (MFB) from closest BLASTn http://blast.ncbi.nlm.nih.gov/Blast) match with accession numbers, maximum identity (%) and query coverage (%) values. Bacterial categories classified by biological characteristics: op. path., opportunistic pathogens; sap., saprophyte; path., strict pathogen; symb., symbiont. One bacterium (MFB1) had matches below query threshold so could not be definitively identified by this method.

Phylum	MFB	Closest BLASTn match (name, accession no,)	Max identity (%) (Query coverage %)	Bacterial category
Actinobacteria	1	*Micrococcus luteus, AB088764.1*	82 (47),	sap./op.path
	2	*Micrococcus luteus, AB088764.2*	98 (60)	sap./op.path
Firmicutes	3	*Bacillus cereus, EU871042.1*	99 (100)	op.path
	4	*Carnobacterium mobile AF374289.1*	71 (83)	sap.
	5	*Staphylococcus aureus,* U39769.1	100 (59)	op.path
Beta-proteobacteria	6	*Alcaligenes faecalis EU014606.1*	96 (90)	op.path
Gamma-proteobacteria	7	*Escherichia coli FJ823387.1*	96 (55)	op.path
Uncultured bacteria	8	Uncultured bacterium O1_44 FJ356614.1	89 (74)	symb.
	9	Uncultured bacterium O1_44 FJ356325.1	95(68)	symb.

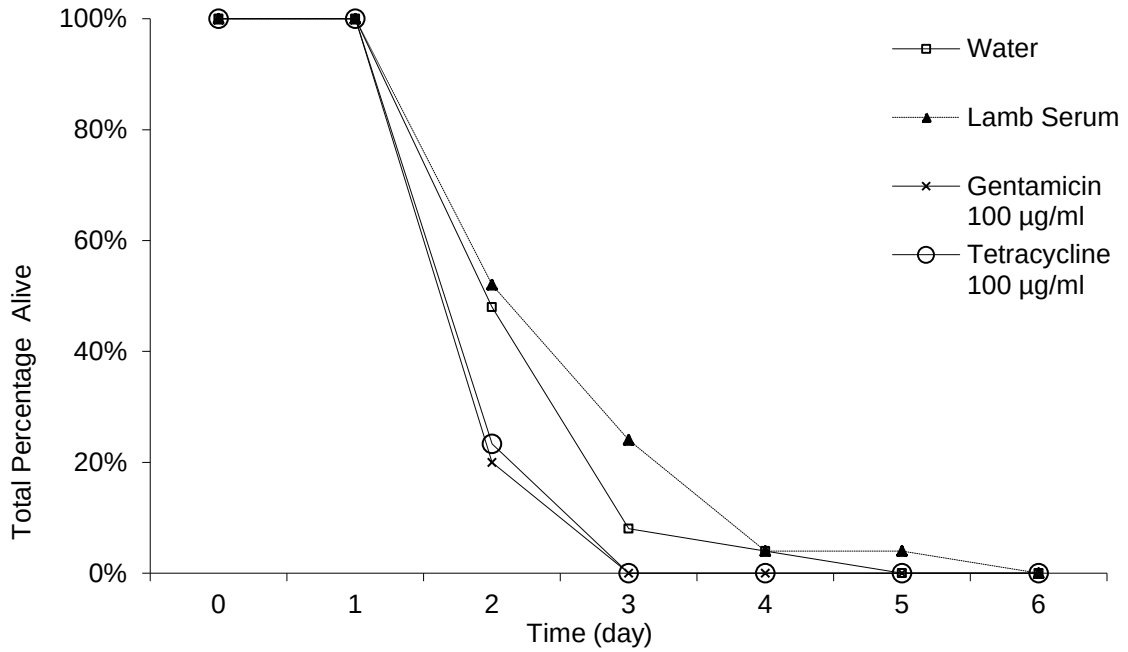

Figure 1. Survival curves for *P. ovis* mites fed gentamicin (100 µg/ml) and tetracycline (100 µg/ml). Significant difference of curves (LR_4 = 23.12, *P* <0.001). Five chambers per treatment with total number of mites at start of experiment (n=100).

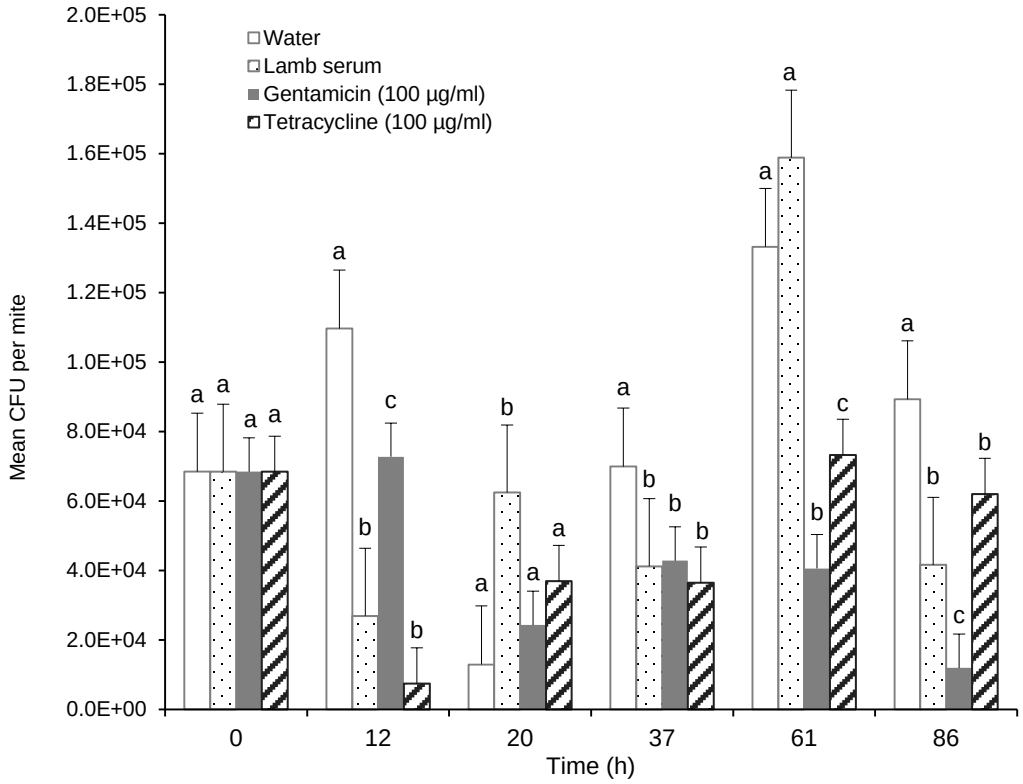

Figure 2 Effect of antibiotics on bacterial density in *P. ovis* mites. Significant effect of antibiotic treatment (F_1=4.67, *P*=0.039) on mean bacterial density (CFU/mite) but no significant effect of time (F_4=2.15, *P*=0.099). Error bars are SEM, N =3 per treatment. Letters above error bars indicate significant differences between treatments groups within time points.