

Dear Colleagues

Welcome to the 1st International Conference on MRSA in Animals – Epidemiology and Infection, hosted by the University of Liverpool, in association with the Bella Moss Foundation.

Welcome also to Leahurst, the University of Liverpool's veterinary field station, which also houses the equine and farm animal hospitals and in 2007 our brand new small animal hospital. Leahurst is situated in a very pleasant, green area of the West Wirral peninsula and we hope you will enjoy your stay with us.

The aim of this conference is to bring together researchers from the veterinary and human field working on MRSA, as well as clinicians, to disseminate and further our knowledge on the epidemiology and infection of MRSA in animals.

The conference would not have been possible without the commitment of our speakers, our generous sponsors, the local organising team and the Bella Moss Foundation. In particular we must thank Gill Beckett and Sheila Nugent for their tireless help in putting the conference together and ensuring its smooth running.

On behalf of the organising committee, we would like to thank all the participants for their contributions to this conference.

A very warm welcome to you all.

Susan Dawson and Nicola Williams

Local Organising Committee

Dr S Dawson

Dr N Williams

Local Administration

Mrs S Nugent

Mrs G Beckett

The Bella Moss Foundation

Ms J Moss

Mr M Doshier

General Information

Badges

Badges must be worn at all times whilst on the Leahurst site. For identification purposes, we would be grateful if badges could be worn during the dinner at Thornton Hall on 21st June.

Banks

The nearest bank is situated in Neston – please consult the Receptionist for directions.

Car Parking

There will be ample parking at Leahurst for delegates travelling by car.

Catering

All refreshment and lunch breaks will be taken in the marquee.

Certificate of Attendance

A certificate of attendance is included with your registration documents.

Toilets & Cloakroom

Toilets are located adjacent to the Reception area of the Main Building. A cloakroom will be available during the conference for luggage and other belongings. This will be situated in a room adjacent to the Reception area.

Email

Delegates will have access to computers in order to access emails.

Taxis

Local taxi firms are:
Riverside Taxis - 0151 336 1555
Thunderbird Taxis - 0151 336 3999

Telephones

There is a public telephone located next to the staircase in the foyer of the Main Building.

Language

The official language of the symposium will be English. No simultaneous translation is available.

Messages

Messages for delegates should be handed in at the Registration Desk in the foyer of the Main Building. Telephone and fax messages may be left for delegates during office hours on the following number: 0151 794 6016. Messages will be posted on the notice board adjacent to the Registration Desk.

First Aid

Please contact either the Receptionist or the Admin Office in the Main Building for any first aid requirements.

Lost Property

Enquiries regarding items lost or found should be directed to the Receptionist in the Main Building.

Smoking Policy

Smoking is not permitted throughout the Leahurst site or in the Marquee.

Disclaimer

All best endeavours will be made to present the programme as printed. However, we reserve the right to alter or cancel, without prior notice, any arrangements, timetables, plans or other items relating directly or indirectly to the Congress for any cause beyond its reasonable control. We do not accept liability for any loss or inconvenience caused as a result of such cancellation.

Social Programme

Welcome Reception

Monday 19th June 2006
18.30 – 22.00

- Registration will be in the foyer area of the Main Building at Leahurst.
- A buffet reception will take place during the evening in a Marquee situated in the grounds of Leahurst House.
- A cloakroom will be available in a room located off the Reception area for coats and luggage, etc.
- Taxis to hotels can be arranged by the Reception staff on request.



Conference Dinner at Thornton Hall

Tuesday 20th June 2006
19.00 – 23.00

- The conference dinner will be held in the Torintone Suite at Thornton Hall Hotel, Thornton Hough.
- Reception drinks at 19.00 for dinner at 19.30.
- Hotel details are included in your delegate pack.



Scientific Programme

Monday 19th June 2006

18.00 – 19.00	Registration: Main Reception, Leahurst
19.00 – 22.00	Social Evening with Buffet at Leahurst

Tuesday 20th JUNE 2006

	09.20 – 09.30	Welcome	Dr Susan Dawson & Ms Jill Moss
1A	09.30 – 10.20	MRSA in Small Animal Medicine – An Overview	Prof David Lloyd
1B	10.20 – 11.00	MRSA infection and colonization in horses	Dr Scott Weese
	11.00 – 11.30	Coffee	
1C	11.30 – 12.10	Risk factors for MRSA infections in dogs and cats: case control study	Dr Anette Loeffler
1D	12.10 – 12.40	MRSA control in veterinary practice	Dr Tim Nuttall
1E	12.40 – 13.10	MRSA in Farm Animals	Dr Chris Teale
	13.10 – 14.10	Lunch	
1F	14.10 – 14.50	Evolution, MLST and MRSA	Dr Mark Enright
1G	14.50 – 15.30	MRSA in animals – an Irish experience	Dr Nola Leonard
1H	15.30 – 16.00	Diagnostic approaches	Mr Bob Graham
	16.00 – 16.20	Coffee	
	16.20 – 17.20	Open session for short papers (of 20 minutes each)	
1J		Characterisation of MRSA in companion animals: a Scottish perspective	Dr Donald Morrison
1K		Emergence of MRSA infections in horses in a veterinary hospital: strain characterization and comparison to MRSA in humans	Ms Christiane Cuny
1L		MRSA colonization in veterinary professionals	Dr Beth Hanselman
	17.20 – 17.45	Discussion and close of meeting	
	19.30 – 23.00	Dinner at Thornton Hall Hotel	

Speakers for individual scientific presentations will be selected from submitted abstracts.

Wednesday 21st June 2006

2A	09.00 – 09.40	Molecular epidemiology of MRSA in the UK	Dr Angela Kearns
2B	09.40 – 10.20	MRSA – Current situation in human hospitals	Prof Tony Hart
2C	10.20 – 10.50	Typing of MRSA, the future: VNTR analysis	Prof Peter Hawkey
	10.50 – 11.20	Coffee	
2D	11.20 – 11.50	Spa typing of MRSA isolated from animals and veterinary staff	Dr Luca Guardabassi
2E	11.50 – 12.20	MRSA and microarrays	Dr Jodi Lindsay
2F	12.20 – 12.50	Molecular epidemiology of MRSA in animals	Dr Nicola Williams
	12.50 – 13.00	Discussion	
	13.00 – 14.00	Lunch and poster session	
	14.00 – 15.30	Short papers	
2G		Concordance of cluster analyses based on spa-typing, MLST and Sma1-macrorestriction analysis - application to veterinary isolates	Dr Birgit Strommenger
2H		Multi locus sequence typing (MLST) and cassette chromosome mec (SCCmec) characterisation of MRSA isolated from animals and veterinary personnel in Ireland	Dr Rebecca O'Mahony
2J		Rapid, reliable sub-typing of MRSA by denaturing high pressure liquid chromatography and DNA sequence analysis	Ms Francine Jury
	15.30 – 16.00	Panel discussion chaired by DEFRA: Where do we go from here?	
	16.30	Close	

Scientific Programme

1A MRSA in Small Animal Medicine – an Overview

David H. Lloyd, Department of Veterinary Clinical Sciences, Royal Veterinary College, Hawkshead Campus, North Mymms, Hertfordshire.

The principal pathogenic *Staphylococcus* of domestic pets is *S.intermedius* but *S.aureus* is a cause of significant infections in dogs and cats, and has generally presented a greater challenge to antimicrobial treatment in pets on account of its broader resistance spectrum (Hoekstra & Paulton 2002). With the recognition of methicillin-resistant *S.aureus* (MRSA) as a cause of infection in animals this challenge has become much more significant. Veterinary practitioners are now obliged to consider more carefully 1) the possibility that animals they treat may be carriers or infected with MRSA, 2) the consequences this may have for treatment of affected animals and 3) the risks of transfer to other animals and to staff.

MRSA infection is now recognised as a problem in both companion animals, including horses, and in farm animals. This review will focus on MRSA infection and carriage in small animals.

The first reports of MRSA in pets described carriage rather than infection. Nasal carriage was first reported in two Nigerian dogs in 1972 (Ojo 1972). Carriage by a ward cat in the UK was associated with recurrent MRSA infection amongst in-contact patients in 1988 (Scott *et al.* 1988) and in 1994 infection amongst healthcare workers was linked to carriage by their dog (Cefai *et al.* 1994). A report from Brazil in 1998 described isolation of MRSA from the skin of three of 148 normal cats (Lilenbaum *et al.* 1998).

Canine infection was first reported in 1999 (Tomlin *et al.* 1999). Retrospective analysis of 11 animals in North America and the UK showed infection was associated with surgical treatment, especially orthopaedic surgery, but infection following trauma and in recurrent pyoderma was also seen. In the British Isles, reports in 2004, provided warning that MRSA was becoming a problem in small animals. Rich and Roberts (2004) reported isolation of 95 MRSA from specimens submitted to a veterinary laboratory during 2003. In March 2004, Boag *et al.* (2004) reported an increase in cases of MRSA infection seen at a small animal referral hospital; 12 cases had been confirmed in dogs and cats over the previous 5-months. A review of MRSA in dogs and cats in 2004 described it as an emerging problem (Duquette & Nuttall, 2004).

Since 2000 there have been increasing numbers of reports of MRSA in animals including over 30 published articles from the US, Canada, Netherlands, Germany, Switzerland, the UK, Korea and Japan. Most of these have dealt with infection

and or carriage by dogs and cats, although there are now substantial numbers of reports of infection in horses and other species including a rabbit, a seal (O'Mahoney *et al.* 2005) and birds (Kitai, 2005).

The increasing recognition of MRSA infection in small animals presents problems for veterinary clinicians relating to restriction of treatment options, and the need to prevent cross-infection amongst inpatients and acquisition of MRSA by staff. However, in studies of infection and carriage of MRSA at the Royal Veterinary College (Loeffler *et al.* 2005), isolates from animals and the environment of the referral hospital were all sensitive to potentiated sulphonamides, oxytetracycline and fusidic acid; a similar sensitivity pattern has been observed in other reports (van Duijkeren *et al.* 2004) and thus effective antimicrobial treatment can usually be instituted.

The need for early recognition of infected animals and their isolation and barrier nursing presents more difficult problems. Dogs and cats in veterinary care commonly require antimicrobial therapy. Such therapy will usually act against *S.intermedius* and resident strains will be removed (Loeffler *et al.* 2005). Animals that are susceptible to staphylococcal infection will thus be particularly at risk to resistant organisms such as MRSA. This risk is increased with *S.aureus* by its ability to survive for very long periods (over 300 days) even in dry environments (Wagenvoort *et al.* 2000). There is an urgent need to increase the awareness of veterinarians to these issues so that they can recognise risks posed by MRSA and take appropriate precautions. In the UK, BSAVA has taken a lead in providing such advice and appropriate documentation is available on the internet for BSAVA members (www.bsava.co.uk).

It is clear that MRSA is becoming a worldwide veterinary problem. The principal sources of infections in pets seem to be infected humans or exposure to medical premises and healthcare staff. Where isolates have been examined for relatedness to human epidemic clones, they have nearly always been closely related. In the UK, they have been shown to be related to EMRSA-15 or EMRSA-16, which are responsible together for over 90% of *S.aureus* bloodstream infections in UK hospitals (Johnson *et al.* 2001). Similar situations have been demonstrated in other countries. It is recognised that transfer of pathogenic staphylococci occurs between pets and humans in close contact, including both veterinary surgeons and owners (Loeffler *et al.* 2004, Manian 2003, O'Mahony *et al.* 2005). Indeed, there is increasing evidence that veterinary staff may be particularly at risk to such transfer and detailed studies of the relevant risk factors are required.

Despite the observation that most pet isolates are closely related to human epidemic clones, some studies have identified isolates from pets are distinct from those found in man (Enoch *et al.* 2005) and it is not known whether dogs and cats can carry their own strains and whether transmission occurs amongst pet animals. Most studies have focused thus far on infected animals and studies of healthy animals have

nearly always depended on single sampling occasions and thus demonstrate carriage but do not confirm colonisation. There is a need now to study larger populations of healthy pets to determine their status as carriers of MRSA and also to set baselines against which any changes in the frequency of MRSA colonisation and infection amongst pets, owners and veterinary staff may be measured.

In human medicine, certain countries have controlled MRSA infection effectively, notably, in Europe, the Netherlands and Scandinavian countries. This has been achieved by instituting measures that control risk factors promoting such infection. There is an urgent need for the veterinary profession to take action now to identify such risks as they apply to domestic animals and devise measures that will limit MRSA carriage and infection in animals.

References

- Boag AK, Loeffler A, Lloyd DH. Methicillin-resistant *Staphylococcus aureus* in small animal practice. *Veterinary Record* 2004; 154: 411.
- Cefai C, Ashurst S, Owens C. Human carriage of methicillin-resistant *Staphylococcus aureus* linked with a pet dog. *The Lancet* 1994; 344: 539-540.
- Duquette RA, Nuttall TJ. Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? *Journal of Small Animal Practice* 2004; 45: 591-7.
- Enoch DA, Karas JA, Slater JD, Emery MM, Kearns AM, Farrington M. MRSA Carriage in a pet therapy dog. *Journal of Hospital Infection* 2004; 60: 186-8.
- Hoekstra, KA, Paulton RJL. *Clinical prevalence and antimicrobial susceptibility of Staphylococcus aureus and Staphylococcus intermedius in dogs.* *Journal of Applied Microbiology* 2002; 93, 406-13.
- Johnson AP, Aucken HM, Cavendish S, Ganner M, Wale MC, Warner M, Livermore DM, Cookson BD, UK EARSS participants. Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS). *Journal of Antimicrobial Chemotherapy* 2001; 48: 143-4.
- Kitai S, Shimizu A, Kawano J, Sato E, Nakano C, Uji T, Kitagawa H. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. *Journal of Veterinary Medical Science* 2005; 67: 107-10.
- Lilenbaum W, Nunes EL, Azeredo MA. Prevalence and antimicrobial susceptibility of staphylococci isolated from the skin surface of clinically normal cats. *Letters of Applied Microbiology* 1998; 27: 224-228.
- Loeffler A, Boag AK, Sung JM, Lindsay JA, Guardabassi L, Dalsgaard A, Smith H, Stevens KB, Lloyd DH. Prevalence of

methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. *Journal of Antimicrobial Chemotherapy* 2005; 56: 692-697.

Ojo MO. Bacteriophage types and antibiotic sensitivity of *Staphylococcus aureus* isolated from swabs of the noses and skins of dogs. *Veterinary Record* 1972; 91: 152-3.

O'Mahony R, Abbott Y, Leonard FC, Markey BK, Quinn PJ, Pollock PJ, Fanning S, Rossney AS. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. *Veterinary Microbiology* 2005; 109: 285-96.

Rich M, Roberts L. Methicillin-resistant *Staphylococcus aureus* isolates from companion animals. *Veterinary Record* 2004; 154: 310.

Saijonmaa-Koulumies LE, Lloyd DH. Colonisation of the canine skin with bacteria. *Veterinary Dermatology* 1996; 7: 153-63.

Scott GM, Thomson R, Maloney-Lee J, Ridgway GL. Cross-infection between animals and man: possible feline transmission of *Staphylococcus aureus* infection in humans? *Journal of Hospital Infection* 1988; 12: 29-34.

Tomlin J, Pead MJ, Lloyd DH, Howell S, Hartmann F, Jackson HA, Muir P. Methicillin-resistant *Staphylococcus aureus* (MRSA) infection in 11 dogs. *Veterinary Record* 1999; 144: 60-64.

Van Duijkeren E, Box ATA, Heck MEOC, Wannet WJB, Fluit. Methicillin-resistant staphylococci isolated from animals. *Veterinary Microbiology* 2004; 103: 91-7.

Wagenvoort JH, Sluijsmans W, Penders RJ. Better environmental survival of outbreak vs. sporadic MRSA isolates. *Journal of Hospital Infection* 2000; 45: 231-4.

1B Methicillin-resistant *Staphylococcus aureus* infection and colonization in horses

J Scott Weese DVM DVSc DipACVIM
Dept of Clinical Studies, Ontario Veterinary College
University of Guelph, Guelph, Ontario, Canada

Methicillin-resistant *S. aureus* (MRSA) is an emerging problem in horses internationally. A variety of different issues are of concern, including morbidity and mortality in horses, pressure to use different antimicrobials in horses, and risks to humans in contact with infected or colonized horses.

Initial reports of MRSA infections in horses were case reports or limited case series. Hartmann *et al.* reported a post-operative MRSA wound infection in a horse at a veterinary hospital in the United States, and speculated that it was caused by a human source.¹

The thought that equine MRSA was an aberrant and uncommon effect of direct infection of horses by colonized personnel was also present in a subsequent report of MRSA infections in 11 horses over 13 months in another American veterinary teaching hospital, and was strengthened by isolation of indistinguishable MRSA isolates from three hospital personnel.² All horses developed infections at the sites of therapeutic intervention, including surgery, joint invasion and vaccination.

While there were anecdotal reports of further MRSA infections at equine hospitals, including outbreaks, a few years passed before there were reports of larger numbers of affected animals. A study at a veterinary teaching hospital in Canada reported isolation of MRSA from 79 horses from 2000 to 2002.³ Thirteen of these horses had clinical MRSA infections, while the remaining horses were colonized in their nasal passages. Both hospital- and community-associated infections were identified, and there appeared to be ready transmission between horses and horse personnel. Twenty-four clinical MRSA infections were reported in Austrian horses between 2003 and 2005.⁴ Eleven horses were identified with MRSA at a equine hospital in the United Kingdom, including 3 clinical infections.⁵ O'Mahony et al recently reported MRSA infections in 8 horses in Ireland.⁶ A study involving six diagnostic laboratories in the United States reported that 4/18 submitted *S. aureus* isolates from clinical specimens were MRSA.⁷

The spectrum of clinical infections in horses is what would typically be expected with a pathogen such as *S. aureus* and is similar to reports of community-associated MRSA infections in humans.⁸ Skin and soft tissue infection, particularly post-operative and wound infections, have predominated in reports of equine infections.^{1-4,6} Bacteremia/septicemia, pneumonia, surgical implant infection, septic arthritis, skin infections, osteomyelitis, metritis and omphalophlebitis have also been reported.^{3-5,9} At this point, data are insufficient to determine whether MRSA infections in horses are associated with increased morbidity or mortality compared to infections caused by methicillin-susceptible *S. aureus* or other organisms.

The first broad community-based investigation of MRSA in horses was performed in Canada and the United States. Nasal MRSA colonization was identified in 46/972 (4.7%) of horses, yet colonized horses were exclusively from farms with a history of previously infected or colonized horses.¹⁰ The zero prevalence in horses from farms without a history of prior MRSA infection or colonization suggested that there might be a close link between amplifying sites (ie veterinary hospitals) and farms, with subsequent dissemination on farms but little true community-associated transmission between farms. A subsequent study of horses upon admission to a Canadiano veterinary teaching hospital, however, identified MRSA colonization in 2.7% of horses, many of which originated from farms with no history of previous MRSA exposure and no previous contact with a veterinary hospital, suggesting that MRSA may be disseminated in the community.¹¹ Baptiste et al also postulated that MRSA is

present in the general horse population based on their identification of MRSA infections in horses from different sources.⁵

Currently, the prevalence of MRSA colonization in most areas is likely low at this time. Prevalence studies of healthy horses in the Netherlands (n=200) and Slovenia (n=300) reported no MRSA colonization,¹² (M Vengust et al, unpublished data) and a study of 20 *S. aureus* isolates from horses in the Netherlands also reported no MRSA.¹³ Similarly, no colonized horses were identified in a survey of 40 horses from the community in the United Kingdom.⁵

Hospital Screening

While routine screening for MRSA colonization is commonplace in many human hospitals, it is uncommonly performed in equine hospitals. At the Ontario Veterinary College, nasal swabs are collected from horses at the time of admission, weekly during hospitalization, and at the time of discharge. From 2002-2004, the admission colonization incidence rate was 27/1000 admissions, while the hospital-associated colonization and infection incidence rates were 23/1000 and 1.8/1000, respectively and the hospital-associated infection incidence density was 0.88/1000 patient-days.¹¹ Since 2004, the community-associated and hospital-associated rates have dropped dramatically, potentially because of community-based eradication measures and aggressive hospital infection control precautions.

Cuny et al reported an MRSA infection incidence rate of 4.8/1000 equine admissions, at a veterinary teaching hospital in Vienna.⁴ This was based on passive surveillance and it is unclear whether these were predominantly hospital-associated or community-associated.

Diagnosis

Diagnosis of MRSA infection involves standard culture and susceptibility testing of clinical samples. Identification of colonization is somewhat different and more variable. Most screening studies have involved nasal swabs. While a thorough investigation of multisite colonization has not yet been reported, nasal swabs appear to be a reasonable choice. The author initially tested both nasal and rectal swabs, as well as swabs from other body sites, and while MRSA was sometimes isolated from rectal swabs, no situations were encountered where MRSA was not isolated from the nose, but was isolated from another colonized body site. (JS Weese, unpublished data).

The use of enrichment culture, alone or in combination with direct culture, may increase the sensitivity of screening. One study reported a significantly higher recovery of MRSA from nasal swabs from horses, but not humans, using an enrichment procedure compared to direct inoculation onto mannitol-salt agar with 2ug/ml oxacillin.¹⁰ A variety of other screening media are available, yet there has been little

objective evaluation in horses. Further studies may identify optimal techniques for screening.

In humans, the use of real-time PCR for rapid identification of MRSA colonization has been proposed as a useful tool because of the ability to receive results within hours instead of days. A commercial real-time PCR assay has been validated in humans,¹⁴ and is being used clinically in some human hospitals. Unfortunately, this assay does not appear to be useful in horses because of a high rate of undetermined results and a high false-positive rate.(MEC Anderson et al, unpublished data)

Some idiosyncracies in the predominant MRSA clone affecting horses in North America have been reported, which can complicate testing. This clone, Canadian epidemic MRSA-5 (USA500, eMRSA5, ST8:MRSA:IV) is poorly coagulase positive and often appears coagulase negative using a tube coagulase test, and therefore could be missed if other testing such as *S. aureus* LAT is not performed. Additionally, *in vitro* oxacillin resistance is often delayed or inducible, and isolates may appear susceptible at 24 hours yet resistant after 48 hours. This appears to be particularly problematic with some automated antimicrobial susceptibility testing systems. If screening plates containing oxacillin are only read after 24 hours, some isolates could be missed as no growth is sometimes evident after 24 hours with heavy growth apparent 24 hours later. Cefoxitin is a better inducer of *mecA* and may be a better supplement for screening media, yet objective comparisons of screening media for equine samples has not been reported.

Molecular Epidemiology

Lack of international standard for classification of MRSA isolates, and regional differences in methodology of certain typing methods somewhat hampers characterization of MRSA internationally. PFGE and MLST are most often used, while other methods such as *spa* typing and *SCCmec* typing are also commonly applied. While MLST nomenclature is standardized, PFGE clone nomenclature varies between countries.

In North America, one clone, termed Canadian epidemic MRSA-5 (CMRSA-5), also known as USA500 and eMRSA5, has accounted for greater than 97% of isolates obtained from colonized and infected horses, and horse personnel.^{3,10,11,15,16} This is a human epidemic clone, yet it is uncommon in people, accounting for less than 5% of human isolates, so the predominance of this clone suggests that it is somehow more adept at colonizing and/or infecting horses. CMRSA-5 is of sequence type 8, possesses *SCCmecIV*, does not carry genes encoding for Pantone-Valentine leukocidin (PVL) production and is multidrug resistant. There is some evidence the CMRSA-5 is also present in horses in other countries. A report of MRSA infection in eight horses in Ireland stated that seven possessed an indistinguishable pattern that was different from non-equine animals and a database of isolates from people.⁶ However, three of these isolates were subsequently identified

as CMRSA-5 by PFGE. This clone has also been identified in equine veterinarians from Ireland, the United Kingdom and Denmark.(unpublished data) The reason for the apparent predilection of CMRSA-5/USA500 in horses is currently unclear. An initial hypothesis was that this clone might be closely related to commensal MSSA or horses. However, a preliminary study of healthy horses in Ontario did not identify ST8 MSSA, or MSSA with other similar sequence types as part of the commensal microflora (MEC Anderson et al, unpublished data).

While CMRSA-5 has predominated in certain areas, it is currently unclear whether this is true in all countries, as limited reports from the United Kingdom and Europe have not reported the same phenomenon. One study of 12 equine isolates from the United Kingdom reported that PFGE patterns were not consistent with major UK epidemic clones or CMRSA-5.⁵ An Austrian study reported that MRSA isolates from horses were not consistent with national human clones.⁴ It is interesting to note, however, that the horse isolates in this study were ST254, which only differs from ST8 by one allele (3,32,1,1,4,4,3 vs 3,3,1,1,4,4,3) and had similar antimicrobial susceptibility pattern to that of CMRSA-5, notable tetracycline and trimethoprim-sulfa resistance and fluoroquinolone susceptibility.

One finding that is consistent internationally is the predominance of strains carrying *SCCmecIV*.³⁻⁵ Genes encoding for PVL production have not yet been reported in equine isolates.

Antimicrobial susceptibility

Variable susceptibility patterns may be present with MRSA strains from the same clonal lineage, however there are often certain markers for particular clones. In North America, a pattern of moderate antimicrobial resistance, including tetracycline and trimethoprim-sulfa resistance and frequent fluoroquinolone susceptibility, has predominated in conjunction with the dominance of CMRSA-5.^{3,10} All tested North American isolates have been susceptible to mupirocin, linezolid, quinupristin-dalfopristin and vancomycin. Variable erythromycin resistance has been reported, as has inducible clindamycin resistance.³ Tetracycline resistance with fluoroquinolone susceptibility is an uncommon pattern, and can be used to suggest the presence of CMRSA-5. O'Mahony et al reported that unlike isolates from other species, equine MRSA isolates (some of which were subsequently identified as CMRSA-5) were all resistant to aminoglycosides, tetracyclines, fluoroquinolones and 7/8 were resistant to trimethoprim-sulfamethoxazole.⁶ All equine isolates from a British study were resistant to gentamicin, with common resistance to rifampin (80%), ciprofloxacin (78%), fusidic acid (69%), co-trimoxazole (50%) and tetracycline (50%).⁵ A study of 5 equine isolates from Austria reported resistance to tetracycline, gentamicin and trimethoprim sulfa.⁴

Risk Factors

An understanding of risk factors for community-associated and hospital-associated MRSA colonization and infection is important to help clarify the epidemiology of disease and to assist in the development of control programs. To date, there has been minimal evaluation of risk factors and this has focused on horses in North America. It is important to realize the limitations of risk factors analyses, and to not over-apply the results. Risk factor studies only identify risk factors for that defined study population. Extrapolation of results to broader populations may be reasonable in many situations, but considering the international epidemiology of MRSA in horses in unclear and may be geographically variable, repeated studies of different populations are required.

Risk factors for community and hospital acquired MRSA infection and colonization have been reported. Farm size (greater than 20 horses) was the only reported risk factor in a study of colonization of horses in the community in Canada and the United States.¹⁰ However, prior antimicrobial administration (within 30 days), previous colonization (within 1 year), previous identification of infected or colonized horses on the farm (within 1 year), admission to the neonatal intensive care unit and admission to the internal medicine service were risk factors for colonization at the time of admission to a Canadian veterinary teaching hospital (JS Weese and S Lefebvre, unpublished data). Administration of ceftiofur or aminoglycosides during hospitalization were risk factors for hospital-associated MRSA colonization in a similar study.¹¹

Interspecies/Zoonotic Transmission

The potential for transmission of MRSA between horses and humans, in both directions, has raised significant concern. There are various reports of nasal colonization of veterinary personnel, and concurrent screening of horses and horse personnel at the same facilities have reported colonization of both species with indistinguishable strains.^{2-4,6,10,15} A study of veterinary and farm personnel in Ontario, focused on an outbreak situation, reported colonization in 27 horse personnel at a veterinary hospital and on private horse farms.³ A subsequent study of horse personnel on farms in Ontario and New York state reported colonization with CMRSA-5 in 12% of sampled individuals.³ Of concern, on every farm with a colonized horse there was at least one person colonized with an indistinguishable strain. Cuny et al reported colonization with ST254 MRSA in 2/43 (5%) personnel at an Austrian equine hospital.⁴ Four veterinary personnel working with 8 infected horses in two veterinary practices in Ireland were colonized.⁶ Three of these isolates were subsequently identified as CMRSA-5 (unpublished data).

It is possible that MRSA colonization and infection are occupational risks in veterinary medicine, particularly amongst equine personnel. A study of veterinarians attending an international conference in the United States in 2005 isolated MRSA from 6.5% of veterinary personnel, including

13.8% of equine veterinarians (Hanselman et al, unpublished data). This prevalence of colonization was striking, and it was hypothesized that occupational exposure was involved as CMRSA-5 accounted for 87% of isolates from equine personnel, including veterinarians from the United Kingdom and Denmark.

There are only 2 reports of clinical zoonotic infection arising from horses. The first was a tattoo-site infection in a veterinarian working with a colonized horse.³ The second involved skin infection in 3 neonatal ICU personnel that worked with an infected foal.¹⁵ Remarkably, infections occurred in otherwise healthy individuals following 4-hour barriered (gloves, coveralls) contact, and overall 19% of personnel that had this type of contact became colonized.

MRSA Control

Control of MRSA infection and colonization are controversial topics in human medicine, and widely varied approaches are taken. While information from human medicine is useful, extrapolation to the veterinary situation should be performed with care because of potential differences in the epidemiology of disease, particularly prevalence of colonization, dynamics of colonization and shedding, types of contacts between horses and between horses and people, and duration of colonization.

It appears that lifelong colonization may not be a concern in horses as it is in humans. Persistent colonization has not yet been reported in horses, and most horses appear to eliminate MRSA colonization within weeks if re-infection is prevented.¹⁶ This is an encouraging finding, and if it is confirmed with further studies, this may be an important factor for MRSA control. Eradication of MRSA colonization on a farm using infection control practices has been reported,¹⁶ however this has not been confirmed in a controlled study. There is currently no evidence that antimicrobial-based decolonization is effective in horses. Considering the concerns regarding further development of resistance and the apparent usefulness of infection control practices, a non-antimicrobial approach has been recommended.^{16,17} Specific components of infection-control based eradication have not been evaluated, so it is impossible to say what, if any, of the components are most useful. Briefly, infection control practices include screening of horses (and ideally humans), cohorting of colonized and non-colonized horses, isolation of colonized horses, improvement in hygiene practices (particularly hand hygiene), cohorting of horse personnel whenever possible so that people working with colonized horses do not work with non-colonized horses, prevention of cross-contamination of items and repeated screening of horses with movement of previously colonized horses into an intermediate area while awaiting a repeated negative swab. Further objective study of eradication methods is needed; yet preliminary results are encouraging.

As MRSA is becoming more common in horses and there is increasing recognition of the risk of hospital-associated infection and zoonotic transmission, hospital-based infection

control protocols need to be developed to address this potential threat. Infection control programs are quite variable and can range from passive surveillance only to active surveillance and MRSA-specific infection control measures. At the OVC-VTH, active screening is a key component of the infection control program. Early identification of colonized horses allows for early implementation of protocols for handling colonized horses, including isolation and increased barrier precautions. Screening also helps differentiate community- versus hospital-associated infections, which is useful for monitoring the effectiveness of infection control measures. Identification of farms with endemic MRSA colonization allows for implementation of additional precautions whenever any horse from the farm is admitted, and also identifies farms that might be willing to implement measures to attempt to eradicate MRSA from their population. Hand hygiene is a critical component of any infection control program, and an emphasis on proper hand hygiene is critical. Ready availability of alcohol-based hand sanitizers is a useful measure to improve compliance. Currently, glove use is mandatory for every horse contact at the OVC-VTH. This was implemented when transmission was occurring within the hospital, and its effectiveness is unclear. It is likely that this policy will be changed so that glove use is only mandatory when contacting high-risk sites (nasal passages, wounds) and high-risk animals. Screening of hospital personnel is a controversial measure in both the human and veterinary healthcare systems. At the OVC-VTH, a protocol is in place whereby hospital personnel can request to be screened at any time. Additionally, wider voluntary screening of hospital personnel can be initiated by the Infection Control Committee and the Occupational Health and Safety Department if there is epidemiological evidence of personnel as a source of infection of animals or if there has been widespread, high-risk contact with an infected or colonized animal. All results are confidential, colonized personnel are referred for decolonization therapy and there is no impact on employment status.

Conclusion

While still uncommon, MRSA appears to be well on its way to becoming widely disseminated in the horse populations, if it has not yet already done so. Because of the ability of MRSA to colonize in horses without producing clinical signs, passive control of MRSA will be less rewarding than active programs designed to identify carriers and reduce the risk of transmission. Better means to control MRSA, both in clinical situations and at the population level, are required. However, given the rapid advances in the knowledge of MRSA in horses over the past few years, there is reason to be optimistic that the impact of MRSA on the horse population, and the accompanying public health risks, can be controlled. To achieve this, the field of veterinary MRSA research must continue to advance to further characterize the emergence of this concerning pathogen in horses and horse personnel, and to objectively evaluate treatment, prevention and infection control measures.

References

1. Hartmann FA, Trostle SS, Klohnen AAO. Isolation of methicillin-resistant *Staphylococcus aureus* from a post-operative wound infection in a horse. *J Am Vet Med Assoc* 1997;211:590-592.
2. Seguin JC, Walker RD, Caron JP, et al. Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. *J Clin Microbiol* 1999;37:1459-1463.
3. Weese JS, Archambault M, Willey BM, et al. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000-2002. *Emerg Infect Dis* 2005;11:430-435.
4. Cuny C, Kuehmerle J, Stanek C, et al. Emergence of MRSA infections in horses in a veterinary hospital: strain characterisation and comparison with MRSA from humans. *Euro Surveill* 2006;11.
5. Baptiste KE, Williams K, Williams NJ, et al. Methicillin-resistant staphylococci in companion animals. *Emerg Infect Dis* 2005;11:1942-1944.
6. O'Mahony R, Abbott Y, Leonard FC, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. *Vet Microbiol* 2005;109:285-296.
7. Middleton JR, Fales WH, Luby CD, et al. Surveillance of *Staphylococcus aureus* in veterinary teaching hospitals. *J Clin Microbiol* 2005;43:2916-2919.
8. Fridkin SK, Hageman JC, Morrison M, et al. Methicillin-resistant *Staphylococcus aureus* disease in three communities. *N Engl J Med* 2005;352:1436-1444.
9. Shimizu A, Kawano J, Yamamoto C, et al. Genetic analysis of equine methicillin-resistant *Staphylococcus aureus* by pulsed-field gel electrophoresis. *J Vet Med Sci* 1997;59:935-937.
10. Weese JS, Rousseau J, Traub-Dargatz JL, et al. Community-associated methicillin-resistant *Staphylococcus aureus* in horses and humans who work with horses. *J Am Vet Med Assoc* 2005;226:580-583.
11. Weese JS, Rousseau J, Willey BM, et al. Methicillin-resistant *Staphylococcus aureus* in horses at a veterinary teaching hospital: frequency, characterization, and association with clinical disease. *J Vet Intern Med* 2006;20:182-186.
12. Busscher JF, van Duijkeren E, Sloet van Oldruitenborgh-Oosterbaan MM. The prevalence of methicillin-resistant staphylococci in healthy horses in the Netherlands. *Vet Microbiol* 2005.
13. van Duijkeren E, Box ATA, Heck MEOC, et al. Methicillin-resistant staphylococci isolated from animals. *Vet Microbiol* 2004;103:91-97.

14. Huletsky A, Giroux R, Rossbach V, et al. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. *J Clin Microbiol* 2004;42:1875-1884.
15. Weese JS, Caldwell F, Willey BM, et al. An outbreak of methicillin-resistant *Staphylococcus aureus* skin infections resulting from horse to human transmission in a veterinary hospital. *Vet Microbiol* 2006;114:160-164.
16. Weese JS, Rousseau J. Attempted eradication of methicillin-resistant *Staphylococcus aureus* colonisation in horses on two farms. *Equine Vet J* 2005;37:510-514.
17. Weese JS. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel. *Vet Clin North Am Equine Pract* 2004;20:601-613.

1C Risk Factors for MRSA Infections in Dogs and Cats: A Case-Control Study

Anette Loeffler, Department of Veterinary Clinical Sciences, Royal Veterinary College, University of London, UK.

In recent years, methicillin-resistant *Staphylococcus aureus* (MRSA), one of the most important pathogens associated with human hospital-acquired infection and lately with community-associated disease, has also caused concern in veterinary medicine. While MRSA infections in companion animals can usually be treated successfully with licensed antimicrobial agents, the zoonotic implications associated with MRSA in companion animals require a structured approach.

It is recognised, that MRSA accounts for about 40% of human *S. aureus* bacteraemia isolates in the UK (Johnson and others 2005). In contrast, the frequency of non-bacteraemic MRSA infections and the extent of MRSA carriage amongst non-hospitalised humans in the UK are unknown. Two relatively small studies indicated carriage rates in the community of less than 2% (Abudu and others 2001, Maudsley and others 2004). However, in view of the high prevalence of MRSA infections in hospitals, variations in carrier site sampling methods and isolation techniques, and the lack of information from larger populations, these figures need to be interpreted with care. Risk factors for MRSA carriage and infection in humans include contact with MRSA carriers, hospitalisation or association with other health care facilities, surgery or other invasive procedures, such as catheter placement or injections, and antimicrobial therapy (Graffunder and Venezia 2002). In addition, medical staff have been shown to have higher rates of MRSA carriage than non-medical staff in several countries (Cesur and Cokca 2004) and there is also evidence emerging that veterinary staff may present a risk group for MRSA carriage (Weese 2004, Seguin and others 1999). The increasing number of reports of MRSA infections in pets and

the concern about MRSA carriage amongst veterinary surgeons led to a cross sectional study sampling veterinary staff and hospitalised animals in a busy UK small animal referral hospital for carriage of staphylococci on a single-day. MRSA was isolated from mucosal carrier sites in 18% of veterinary staff and in 9% of hospitalised dogs (Loeffler and others 2005). The study also showed that animals carrying *S. aureus* did not yield *S. intermedius* concurrently at the same carrier sites. This suggested that a selection process had occurred in those animals favouring the acquisition of *S. aureus* which is otherwise less well adapted to canine and feline mucosae. Transfer of *S. aureus*, which is considered a normal inhabitant of human mucosal carrier sites especially the nostrils, from humans to animals is the most likely explanation for the occurrence of *S. aureus*, including MRSA, in dogs and cats and several studies have provided good evidence for transmission between species (Manian 2003, van Duijkeren and others 2004). However the circumstances which facilitate transmission have not been investigated. While *S. intermedius* remains the predominant organism in staphylococcal infections and at carrier sites in dogs and cats, the perceived increase in MRSA infections in companion animals and the zoonotic implications of staphylococcal disease indicate the need for investigations into the possible risk factors for MRSA infection in companion animals.

Based on the hypothesis that risks for MRSA infection in animals mirror those reported for humans, a case-control study, funded by DEFRA, was designed to investigate the risk factors that predispose dogs and cats to MRSA infection and preliminary results are described here. Cases of *S. aureus* infection in dogs and cats are identified by its isolation from diagnostic clinical samples sent to a commercial veterinary laboratory (IDEXX Laboratories, Wetherby) from veterinary practices in the UK. *S. aureus* is recognised at the laboratory using automated isolation technology and microbiological tests for confirmation. Dogs with MRSA infection and those with methicillin-susceptible *S. aureus* (MSSA) infection serving as control cases are included, with the referring veterinary surgeons' consent. Any connection of human participants with the healthcare sector is investigated using questionnaires. The animals' medical histories are analysed with regard to clinical signs at presentation, treatment procedures and antimicrobial therapy. National Health Service (NHS) Ethics Committee approval has been obtained for this study.

During the first seven months of the study period, 68 MRSA cases and 58 MSSA control cases were enrolled with 255 nasal swab samples from in-contact humans returned up to the time of writing. MRSA was isolated from 19/163 (11.7%) veterinary staff and from 5/92 (5.4%) owners. MSSA was found in 30/163 (18.4%) veterinary staff and in 19/92 (20.7%) owners. Twenty of 140 (14.3%) participants in-contact with MRSA infected pets were positive for MRSA carriage compared with 4/115 (3.5%) positive in-contact humans of control dogs and cats. Contact of human participants with healthcare institutions will be identified from returned questionnaires.

This will help to determine whether animals belonging to human MRSA carriers or non-carriers with NHS-contact are at higher risk of acquiring MRSA than those owned by non-carriers and owners without known contact with the NHS. The study isolates from infected animals and in-contact humans will be typed, compared and further analysed at a molecular level to investigate their relatedness to human epidemic clones and to identify possible distinct genetic patterns developing amongst *S. aureus* in animals after transfer between species.

In conclusion, MRSA and methicillin-susceptible *S. aureus* infections are often identified from clinical infections in dogs and cats in the UK. Preliminary results from the risk factor study indicate that a substantial proportion of veterinary staff are carrying MRSA at mucosal sites while MRSA carriage has also been documented amongst pet owners at higher rates than suggested in the literature. Consequently, rigorous screening for MRSA carriage amongst veterinary staff should be instigated to minimise the risk of veterinary-environment-acquired infection and carriage in pet animals. While the true extent of MRSA carriage amongst pet owners remains unknown and uncontrolled, awareness of owners as a possible reservoir for MRSA acquisition for their pets will assist in recognising *S. aureus* infection early in pets and thereby help to control its spread.

References

- Abudu L, Blair I, Fraise A *et al.* Methicillin-resistant *Staphylococcus aureus* (MRSA): a community-based prevalence survey. *Epidemiol Infect* 2001; **126**: 351-6.
- Cesur S, Cokca F. Nasal carriage of methicillin-resistant *Staphylococcus aureus* among hospital staff and outpatients. *Infect Control Hosp Epidemiol* 2004; **25**: 169-71.
- Graffunder EM, Venezia RA. Risk factors associated with nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials. *J Antimicrob Chemother* 2002; **49**: 999-1005.
- Johnson AP, Pearson A, Duckworth G. Surveillance and epidemiology of MRSA bacteraemia in the UK. *J Antimicrob Chemother* 2005; **56**: 455-62.
- Loeffler A, Boag AK, Sung J *et al.* Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. *J Antimicrob Chemother* 2005; **56**: 692-7.
- Manian FA. Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. *Clin Infect Dis* 2003; **36**: e26-8.
- Maudsley J, Stone SP, Kibbler CC *et al.* The community prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in older people living in their own homes: implications for treatment, screening and surveillance in the UK. *J Hosp Infect* 2004; **57**: 258-62.

Seguin JC, Walker RD, Caron JP *et al.* Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to animal transmission. *J Clin Microbiol* 1999; **37**: 1459-63.

Van Duijkeren E, Wolhagen MJHM, Box AT *et al.* Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. *Emerg Infect Dis* 2004; **10**: 2235-7.

Weese JS. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel. *Vet Clin North Am Equine Pract* 2004; **20**: 601-13.

1D Methicillin Resistant *Staphylococcus aureus* (MRSA): control in veterinary practice

Tim Nuttall
University of Liverpool

Introduction

It is likely that veterinary practices encounter animals colonised or infected with MRSA. Animals probably acquire MRSA from humans, but can also act as reservoirs of infection for in-contact humans. MRSA is of little threat to healthy individuals but those at risk include immunocompromised patients, long term in-patients (especially with catheters, implants etc.), and patients with open wounds, mucosal defects and undergoing major surgery. MRSA can be spread by direct contact or indirectly by poor hygiene and readily contaminates premises. Control measures are therefore vital.

Routine measures to prevent the spread of MRSA (and other infectious diseases)

1. Hand washing and disinfection of surfaces and equipment between patients. Alcohol gel pouches on uniforms and kennels are a visual cue for cleanliness and can be quickly used after handling an animal before touching pens, keyboards etc. It is important to avoid materials at hand touch sites that can't be cleaned, e.g. consider using waterproof keyboards or keyboard covers etc.
2. Wearing simple uniforms/coats etc. that can be laundered on site.
3. Wearing of gloves, disposable aprons, masks, eye protection etc. for contact with body fluids, lesions and other contaminated materials.
4. Cover wounds or skin lesions with waterproof dressings.
5. Rational use of antibiotics to minimise the development and spread of antibiotic resistance.

6. High standards of aseptic technique: minimise theatre staff; sterile gowns, gloves, hats and masks; sterilisation of equipment; and single patient use.
7. High standards of cleaning: clean cages and bedding at least once daily; and thoroughly clean and disinfect everything between patients.
8. Segregation of all waste and contaminated material, careful handling of clinical waste and transport in a sealed bag or container of appropriate strength and colour.
9. Ensure that all staff understand and adhere to infection control. Having specific staff to monitor and enforce control measures and undertake infection control audits is advisable.

Managing patients with MRSA

Detection of MRSA

Staphylococci with oxacillin resistance should be confirmed with identification of *mecA* and *femA* genes (culture, especially Kirby-Bauer, can overestimate resistance). Genetic typing to identify the clone is important in epidemiology. Isolation and typing is best done in an experienced laboratory. Samples should be taken from clinical lesions and/or implants as appropriate. Naso-pharyngeal and perineal swabs will detect persistent mucosal colonisation.

Identification of colonised and/or infected animals

Screening all cases prior to admission is not feasible, especially in first opinion clinics. The prevalence and risk factors for carriage MRSA in healthy dogs and cats is as yet unknown and some asymptomatic carrier animals will be undetected. At present, MRSA should be suspected in:

- Patients from known MRSA positive households or that belong to healthcare workers. A substantial proportion of cases have indirect or direct contact with human healthcare environments, although this has not been noted in the majority of recently reported cases.
- Patients with non-healing wounds.
- Patients with non-antibiotic responsive infections where previous cytology and/or culture indicates that staphylococci are involved.
- Nosocomial or secondary infections, especially in at-risk patients.
- Screening hospitalised cases during their stay and/or prior to discharge may be necessary in an environment where MRSA is endemic and/or there is evidence of transmission in the practice.

Staff should be informed about MRSA cases before admission, although this may not be possible in first opinion practice. Practices, however, should culture suspected cases before referral. If in doubt, take a swab at admission and proceed as though MRSA positive until you have the results.

Admission

Admit known or suspected cases directly into a consultation room to avoid contamination and contagion in the waiting room. The consultation room should be disinfected before it is used again. Movement of infected or suspect patients around the practice and procedures involving them should be minimised, and where possible scheduled for the end of the day. Discharging wounds should be covered with an impermeable dressing. Using trolleys will help minimise contamination of corridors etc. Contact between MRSA patients and other animals and staff should be minimal. The trolley and potentially contaminated rooms or corridors should be disinfected before further use.

Hospitalisation

Staff contact should be limited to what is essential. Staff with skin or mucosal barrier defects or who are immunosuppressed should not nurse MRSA positive animals. Barrier nursing precautions in addition to the general measures above include:

- Isolate MRSA patients as far as possible from other patients. MRSA can directly spread for 2-4m and be further disseminated by mechanical transmission.
- Use disposable gloves, gowns, hats, mask and overshoes. Long hair should be tied back and sleeves rolled up to the elbow. Wear eye protection if there is a risk of splashing or aerosols.
- Strict washing of the hands and forearms after handling the patient. Watches and jewellery that could interfere with the efficacy of washing should be removed.
- Pens/pencils, stethoscopes, thermometers etc. should be used with the affected patient only and then disposed of or disinfected.

Bathing every 2-3 days with an antibacterial wash can reduce mucosal and cutaneous carriage, and the potential for contamination. Bathing, however, may not be possible and increases staff contact.

Before surgery, it may be possible to decontaminate the patient (see below). Bathing with an antibacterial shampoo, covering lesions with impermeable dressings, cleaning lesional and/or surgical sites with 70% alcohol, and intra-nasal mupirocin may also reduce the risk of colonising the surgical site.

Treatment

MRSA is not another word for death. Isolates are frequently sensitive to routine antibiotics including potentiated sulphonamides, tetracyclines, macrolides, fucidin (fusidate or fucidic acid) and mupirocin (not licensed for use in animals). The choice should be based on culture, preferably using the minimum inhibitory concentration to calculate the appropriate dose. Further treatment depends on the nature of the primary problem and may require specialist advice (e.g. removing implants, gentamicin impregnated beads and collagen sponges, activated silver dressings etc.).

Discharged and deceased patients

Patients should be discharged as soon clinically fit. Persistently colonised animals can be treated with an antibacterial shampoo and 2% mupirocin (Bactroban® Nasal) intranasally 2-3 times daily. Treatment should not exceed five days and two courses, and should not be repeated at intervals less than one month. Other antibiotics may be appropriate depending on the sensitivity pattern. Decolonisation of humans is difficult; it often involves 3-5 days intranasal fucidin or mupirocin and chlorhexidine washes, and then three negative screens 2-5 days apart, with repeated cycles if necessary. Treatment results in clearance in 91-99% of patients but re-colonisation rates are up to 26%.

If the animal remains colonised potential risks and precautions must be discussed with the owner. It is unfeasible to screen every in-patient prior to discharge, and it is therefore possible some animals that become persistent carriers during hospitalisation will be undetected. Pre-discharge screening, however, is only a measure of the colonisation rate in the practice and it is uncertain whether this is of much clinical importance in healthy individuals.

If an MRSA positive animal dies, the body must be securely bagged. It should be disposed of by cremation; owners that insist on taking the body should sign an acknowledgement of the potential risk.

Screening staff for MRSA

It is important to differentiate transient carriage from colonisation and persistent carriage. Transient carriage is more common and is most effectively controlled by hygiene measures. Surveillance of staff is controversial and consent, confidentiality, stigmatisation and further action must be addressed. It can also miss transiently contaminated staff, who may still be a source of infection if they fail to observe hygienic precautions. Generally, isolation of MRSA in the morning indicates colonisation, and isolation in the evening but not the morning indicates transient contamination. The number of screening cultures is controversial, but three swabs 5-7 days apart are usually taken.

Screening can help if MRSA becomes an endemic problem, although it is difficult to prove that an infection was acquired in the practice in the absence of pre-admission culture, as

asymptomatic and/or transient carriage is not uncommon and is not necessarily relevant. MRSA-positive staff or owners of MRSA-positive animals should be referred to their doctor.

Environmental monitoring

MRSA can survive up to 12 months in hospital dust, bedding and clothing. Routine environmental screening can therefore be useful to monitor cleanliness; one study showed that of 82-91% of visually clean surfaces only 30-45% were microbiologically clean. There are no microbiological standards for hospitals, but MRSA contamination rates decline where cleaners have been trained in microbiological cleanliness. Floors in particular seem to play little role and hand-touch sites are more important.

Animals taken in hospitals and care homes

Routine screening of animals taken in hospitals and care homes (e.g. PAT and CHATA pets) suffers from the caveats about transient contamination versus colonisation and is not feasible in all situations. These animals should be healthy, however, and grooming, bathing with an antibacterial shampoo, routine hygienic precautions (e.g. hand washing, no licking, using impermeable pads, avoiding contact with bedding etc.) and preventing access to high risk patients should minimise any problems.

Further reading

- BSAVA Guidance notes and FAQs - www.bsava.com; follow 'The Practice' link
- DEFRA - www.defra.gov.uk/animalh/diseases/zoonoses/mrsa.htm
- HPA - www.hpa.org.uk/infections/topics_az/staphylo/menu.htm
- Association of Medical Microbiologists (www.amm.co.uk/newamm/html/public.htm)
- Infection Control Nurses' Association (www.icna.co.uk)
- Centers for disease control and prevention - www.cdc.gov; CDC MRSA guidelines and advice - www.cdc.gov/ncidod/dhqp/ar_mrsa.html
- Duquette, R.A. and Nuttall, T.J. (2004) Methicillin resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? *Journal of Small Animal Practice* **45** 591-597.
- Rich M. Staphylococci in animals: Prevalence, identification and antimicrobial susceptibility, with an emphasis on methicillin-resistant *Staphylococcus aureus*. *British Journal of Biomedical Science* 2005 **62** 98-105.
- Weese JS. Methicillin-resistant *Staphylococcus aureus*: an emerging pathogen in small animals. *Journal of the American Animal Hospital Association* 2005 **41** 150-157.

- Tomlin J, Pead MJ, Lloyd DH, Howell S, Hartmann F, Jackson HA, Muir P. Methicillin-resistant *Staphylococcus aureus* infections in 11 dogs. *Veterinary Record* 1999 **144** 60-64.
- Owen MR, Moores AP, Coe RJ. Management of MRSA septic arthritis in a dog using a gentamicin-impregnated collagen sponge. *Journal of Small Animal Practice* 2004 **45** 609-612.
- Enoch DA, Karas JA, Stater JD, Emery MM, Kearns AM, Farrington M. MRSA carriage in a pet therapy dog. *Journal of Hospital Infection* 2005 **60** 186-188.
- Middleton JR, Fales WH, Luby CD, Oaks JL, Sanchez S, Mnyon JM, Wu CC, Maddox CW, Welsh RD, Hartmann F. Surveillance of *Staphylococcus aureus* in veterinary teaching hospitals. *Journal of Clinical Microbiology* 2005 **43** 2916-2919.
- Nicolle LE, Dyck B, Thompson G, Roman S, Kabani A, Plourde P, Fast M, Embil J. Regional dissemination and control of epidemic methicillin-resistant *Staphylococcus aureus*. *Infection Control and Hospital Epidemiology* 1999 **20** 202-205.
- Weese JS, Rousseau J. Attempted eradication of methicillin-resistant *Staphylococcus aureus* colonisation in horses on two farms. *Equine Veterinary Journal* 2005 **37** 510-514.
- O'Mahony R, Abbott Y, Leonard FC, Markey BK, Quinn PJ, Pollock PJ, Fanning S, Rossney AS. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. *Veterinary Microbiology* 2005 **109** 285-296.
- Dunowska M, Morley PS, Hyatt DR. The effect of Virkon®S fogging on survival of *Salmonella enterica* and *Staphylococcus aureus* on surfaces in a veterinary teaching hospital. *Veterinary Microbiology* 2005 **105** 281-289.
- van Duijkeren E, Box ATA, Heck MEOC, Wannet WJB, Fluit AC. Methicillin-resistant staphylococci isolated from animals. *Veterinary Microbiology* 2004 **103** 91-97.
- Weese JS, DaCosta T, Button L, Goth K, Ethier M, Boehnke K. Isolation of methicillin-resistant *Staphylococcus aureus* from the environment in a veterinary teaching hospital. *Journal of Veterinary Internal Medicine* 2004 **18** 468-470.
- Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B, Low DE. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. *Veterinary Microbiology* 2006 (article in press; published online at <http://www.sciencedirect.com/science/journal/03781135>).

1E MRSA in Farm Animals?

Teale, C.J.

Veterinary Laboratories Agency Shrewsbury, Kendal Road, Harlescott, Shrewsbury, SY1 4HD.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in human medicine in 1961, a few years after the introduction of methicillin. MRSA strains are commonly heterogeneous in their expression of methicillin resistance - large differences are observed in the expression of resistance by individual cells within a population. There are practical difficulties in accurately determining susceptibility to methicillin, therefore recent studies often rely on detection of the altered penicillin-binding protein PBP2' which confers resistance or the *mecA* gene which encodes this protein. Some studies in farm animals have used cloxacillin to detect resistance to the penicillinase-resistant penicillins (a group which includes methicillin, oxacillin, nafcillin and cloxacillin) and the presence of methicillin resistance has not been confirmed by detection of PBP2' or the *mecA* gene. Agents recommended as substrates for detection of MRSA in the medical field traditionally include oxacillin and methicillin, whilst cefoxitin is a more recent recommendation; cloxacillin has been found to be less effective than these antimicrobials. These sorts of considerations may be important when interpreting some of the veterinary literature.

MRSA was first reported in dairy cows with mastitis in Belgium in 1972; subsequent studies revealed MRSA in mastitic milk from 20 different Belgian herds. All of the isolates were considered to be representatives of a single strain, which was thought to be of human origin, based on the phage typing schemes in use at that time. Longitudinal studies indicated that the strain could be isolated in some cases more than a year after primary isolation. Human attendants were not sampled in this study. MRSA has also been reported from cattle with sub-clinical mastitis in Bulgaria. In both these studies, detection of either the *mecA* gene or PBP2' was not performed.

Between May 2001 and April 2003, a range of samples were collected from beef and dairy cattle, pigs and chickens and from meat derived from these animal species in Korea. Sampling took place along the food chain from feedlots, through abattoirs and processing plants to retail premises. 28 *S. aureus* isolates were recovered that were resistant to oxacillin and 15 of these possessed the *mecA* gene. 12 of the isolates originated from milk samples taken from dairy cows (and in 9 of these cases, the milk was mastitic). The remaining three isolates all originated from chickens (from two cases of arthritis and a single suppurative muscle lesion). No MRSA isolates were recovered from pigs. Random amplified polymorphic DNA analysis (RAPD) was used to examine the relatedness of these strains to strains from humans and six isolates from cattle gave identical patterns to a human isolate. However, in a further study which looked at 75,335 quarter milk samples from various provinces in Korea, 14 MRSA isolates were recovered and these were shown to possess a unique SCCmec sub-type.

Further typing showed that all of these bovine isolates in this study were related and probably comprised a single strain. The molecular typing suggested that the bovine strains were probably not involved in the emergence of community-acquired MRSA infections in humans in Korea as there were significant differences at the molecular level from current human strains.

Recent reports from the Netherlands have described the detection of MRSA in pigs and humans in-contact with pigs. Pigs were initially suspected as a possible source of MRSA following pre-operative screening of a young patient which had revealed MRSA in both the patient and her parents. The family lived on a pig farm and swabs taken from the nares of 10 pigs did not yield MRSA, although cultures of the perineum of 30 animals did yield a single MRSA isolate from one animal. This isolate and the isolates from the patient and her parents belonged to the same spa-type and were identical by random amplified polymorphic DNA analysis. MRSA isolates which were either identical or differed slightly at the molecular level were recovered at roughly the same time from other people who also had contact with pigs, but who were not apparently linked epidemiologically to the initial case. A group of 26 pig farmers meeting at the index case farm were examined for MRSA and 6 (23% - this figure includes the farm owner) were found to be colonised. A further report has described a case of MRSA mastitis in a mother in Holland, whose baby was also subsequently found to be colonised with MRSA. The husband in this case was a pig farmer and 8 of 10 pigs sampled at random from the herd were found to be carrying MRSA, as were three of four family members and three co-workers on the farm.

References

- Brown, D.F.J., Edwards, D.I., Hawkey, P.M., Morrison, D., Ridgway, G.L., Towner, K.J. and Wren M.W.D. (2005) Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). *Journal of Antimicrobial Chemotherapy* **56**, 1000-1018.
- Devriese, L.A. and Hommez, J. (1975) Epidemiology of methicillin-resistant *Staphylococcus aureus* in dairy herds. *Research in Veterinary Science* **19**, 23-27.
- Thornsberry, C., Caruthers, J.Q. and Baker C.N. (1973) Effect of Temperature on the In Vitro Susceptibility of *Staphylococcus aureus* to Penicillinase-Resistant Penicillins. *Antimicrobial Agents and Chemotherapy* **4**, 263-269.
- Urumova, V., Lyutskanov, M. and Vachkov, A. (2002) Resistance of *Staphylococcus aureus* strains isolated from cattle with sub-clinical mastitis. *Bulgarian Journal of Veterinary Medicine* **5**, 145-152.
- van Dijke, B., Koppen, H., Wannet, W., Huijsdens, X., de Neeling, H. and Voss A. (2006) Methicillin-resistant *Staphylococcus aureus* and pig-farming European Society of Clinical Microbiology and Infectious Diseases Conference

Abstract 16th European Congress of Clinical Microbiology and Infectious Diseases Nice, France, April 1-4 2006 P474 http://www.blackwellpublishing.com/eccmid16/PDFs/cim_1427.pdf accessed 16/5/2006.

Voss, A., Loeffen, F., Bakker, J., Klaassen, C. and Wulf M. (2005) Methicillin-resistant *Staphylococcus aureus* in Pig Farming. *Emerging Infectious Diseases* **11**, 1965-1966.

1F MRSA – Current Situation in Human Hospitals

Prof Tony Hart

1G Methicillin-Resistant *Staphylococcus Aureus* in Animals – An Irish Perspective

F.C. Leonard¹, Y. Abbott¹, A.S. Rossney², R. O'Mahony¹, B.K. Markey¹, P.J. Quinn¹, P.J. Pollock¹.

School of Agriculture, Food Science and Veterinary Medicine¹, University College, Dublin, Belfield, Dublin 4, Ireland

National MRSA Reference Laboratory², St. James's Hospital, Dublin 8, Ireland

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first recognised in Irish hospitals in 1971 (3). Its prevalence increased markedly in the 1990s such that the European Antimicrobial Resistance Surveillance System reported that in 2002, 42% of *S. aureus* isolates from blood cultures in Ireland were methicillin resistant, one of the highest prevalence rates in Europe (1). In addition, infections arising *de novo* in the community are causing increasing concern in many countries and a small number of community-acquired MRSA cases have been described in Ireland (6). The high prevalence of MRSA in humans in Ireland and the close contact between people and their pets provide opportunities for exposure of pets to this human pathogen. This paper provides an overview of the first cases of MRSA infection in animals in Ireland.

The veterinary diagnostic laboratory in University College Dublin (UCD) began testing isolates of *S. aureus* for susceptibility to methicillin (5µg/disk, Oxoid, Ltd.) in mid-2001. Occasional resistant isolates were recognised during the following 12 months but it was not until late 2002 /early 2003 that a cluster of MRSA cases was detected. MRSA was isolated from five dogs which were presented for surgical treatment at a busy urban small animal practice in Ireland between 2001 and 2003. Wound swabs from these cases which were submitted for routine culture and antibiotic susceptibility testing between December 2002 and April 2003, yielded MRSA (4).

Four of the five dogs required surgical intervention for bone fractures following road traffic accidents; one dog required surgical repair of the anterior cruciate ligament. All animals developed post-surgical complications. In most instances, complications occurred within two weeks of initial surgery but in one case the dog was brought to the veterinary practice with a discharging sinus at the surgical site 42 weeks after fracture repair. The five dogs made a complete recovery following removal of all surgical implants and appropriate antimicrobial therapy. Resolution of infection occurred after periods ranging from 12 weeks to greater than one year. The prolonged recovery times are likely to have been due to a number of factors including the presence of implants. In addition, lack of awareness of veterinary staff of the possibility of MRSA infection may have played a part. In two of the five cases, various treatments were attempted for several weeks before wound discharges were sampled for culture and susceptibility testing. Epidemiological typing of the isolates by antibiogram-resistogram typing, biotyping and by chromosomal DNA restriction fragment length polymorphism analysis using *Sma*I digestion followed by pulsed field gel electrophoresis (PFGE), confirmed that the five canine isolates and an isolate from the attending veterinary surgeon were indistinguishable. In addition, these isolates were indistinguishable from the predominant pattern obtained from the most prevalent hospital MRSA strain in the human population in Ireland. The veterinary surgeon was successfully treated for nasal carriage with mupirocin and a number of improvements in infection control procedures were put in place. No further cases of MRSA infection were recorded in this practice during the following two years. Unfortunately, cases of post-surgical wound infections have occurred again recently, possibly due, at least in part, to on-going healthcare associations of staff members in the practice.

The veterinary diagnostic laboratory in UCD continues to isolate MRSA from canine cases with clinical evidence of infection. To date, MRSA isolates have been detected in samples submitted from 21 small animal practices, mostly from post-surgical wounds in dogs. Nasal carriage in attendant veterinary personnel has been detected in almost all practices in which more than one case of MRSA infection has occurred.

The first case of MRSA infection in a horse was detected in an animal referred to the University Veterinary Hospital in September 2003. A further seven equine cases of MRSA infection were identified during the following 12 months. All isolates were obtained from infected wounds and typing data suggested that cross-infection may have occurred within the University Veterinary Hospital in some cases. Isolates from horses and attendant personnel shared similar PFGE and antibiogram-resistogram patterns but these patterns were unlike those exhibited by MRSA seen in human medical practice in Ireland (5). During the first half of 2005, a further nine cases of MRSA infected wounds were identified. All of these cases and one case which had occurred in September 2004, were from the same premises, a large stud farm.

Following a visit by staff of the University Veterinary Hospital, it was clear that the problems being experienced with MRSA infection may have been due to the use of unsuitable accommodation and poor infection control procedures. Some of the buildings were in a poor state of repair, particularly the 'hospital barn' used for housing injured or sick animals and some of the accommodation used for young horses. Difficulties in infection control associated with poor accommodation were compounded by exceptionally large numbers of animals on these premises. Animals were not always separated from others while they were being treated, effective cleaning and disinfection of accommodation was difficult and staff had difficulty maintaining adequate infection control procedures when dealing with large numbers of animals. This is likely to have facilitated spread of MRSA infection and carriage between animals. Nasal carriage of MRSA was detected in one of the attendant veterinary personnel but further molecular typing of the isolate is required to evaluate the importance of this finding. No cases of MRSA infected wounds have been referred to the University Veterinary Hospital from these premises since mid 2005. Improvements in infection control procedures have been put in place and alternative accommodation for young horses was found. Screening of 16 nasal swabs and five wounds from horses in March 2006 failed to yield MRSA.

In Ireland, carriage rates of MRSA in small animals and horses without clinical signs of infection are not known. A study of approximately 300 dogs and cats which all lacked clinical evidence of MRSA infection undergoing procedures requiring a general anaesthetic in the University Veterinary Hospital during the summer of 2005 revealed a carriage rate of approximately 1% (2).

1. Anon. (2003). EARSS Annual Report 2002. RIVM, Bilthoven.
2. Abbott, Y., Leonard, F.C. and Markey, B.K.. (2006). The prevalence of methicillin-resistant *Staphylococcus aureus* infection and colonisation in companion animals in Ireland. These Proceedings.
3. Hone, R., Keane, C.T. (1974). *Infection* **2**:213-217.
4. Leonard, F.C, Abbott, Y., Rossney, A., Quinn, P.J., O'Mahony, R. and Markey, B.K. (2005). *Veterinary Record* **158**, 155-159.
5. O'Mahony, R., Abbott, Y., Leonard, F.C., Markey, B.K., Quinn, P.J., Pollock, P.J., Fanning, S., Rossney, A.S. (2005). *Veterinary Microbiology* **109**:285-296.
6. Rossney Angela, Morgan Pamela, O'Connell Brian, Community-acquired PVL+ MRSA in Ireland: a preliminary report. Euro Surveillance 2005;10(4):E050421.1. Available from: <http://www.eurosurveillance.org/ew/2005/050421.asp#1>

1H Diagnostic Approaches

Mr Bob Graham

1J Characterisation of MRSA in Companion Animals – A Scottish Perspective

D. Morrison¹, G. F. S. Edwards¹, A. Robb¹, D. Taylor² and C. G. Gemmell^{1,3}.

Scottish MRSA Reference Laboratory¹, Glasgow University Veterinary School² and Glasgow University Department of Immunology and Bacteriology³, Glasgow, UK.

The last few years have witnessed an increasing number of reports of MRSA outside the Health Care setting. Characterisation of MRSA isolates causing infection in the community ("Community Associated" MRSA – CA-MRSA) has shown that these are distinct clones from "Hospital Associated" MRSA. The source and reservoir of CA-MRSA is as yet unknown. More recently MRSA in both livestock and companion animals has been reported. In this study we characterised MRSA isolated from companion animals in Scotland to investigate if the source of these MRSA could be determined. Between 2004 and February 2006 27 MRSA were isolated from companion animals by the microbiology laboratory of Glasgow University Veterinary School and characterised by the Scottish MRSA Reference Laboratory (SMRSARL). Thirteen were from dogs, three from cats and 11 were "unknown". Site of infection included wounds, joints, urine and eyes. Twenty six of the 27 MRSA belonged to the "Hospital MRSA" clone EMRSA-15. This clone is the most prevalent in Scotland accounting for 72% of MRSA referred to the SMRSARL. The other MRSA belonged to another "Hospital MRSA" clone, EMRSA-16, which is the second commonest MRSA clone in Scottish hospitals, accounting for 21% of MRSA. PFGE typing divided the 26 E15 into four subtypes (a, b, d and e). The majority (17/26: 65%) of the E15 belonged to a single subtype (E15 subtype b) which is the second commonest E15 subtype among hospital MRSA in Scotland. E15 subtype e accounted for 23% (6/26) and two other subtypes (subtype a and d) were found in 1 and 2 animals, respectively. The SMRSARL has identified 467 E15 subtypes since 1997, however, 80% belong to only 11 subtypes. The four subtypes found in companion animals are in the five commonest subtypes, and account for 60%, of hospital E15. As with hospital acquired EMRSA-15 these isolates were relatively susceptible to antibiotics with the majority being resistant to beta lactams, erythromycin and ciprofloxacin. The single E16 isolate was multi resistant and belonged to a PFGE subtype found in hospitals. Since the largest known reservoir of E15 and E16 is in the health care setting this data suggests that these companion animals would have acquired their MRSA from their owners or from other human "health care" contacts.

1K Emergence of MRSA infections in horses in a veterinary hospital: strain characterization and comparison to MRSA from humans

Christiane Cuny¹, Ewald Denner², Jan Kueimmerle¹, Renate Rosengarten¹, Christian Stanek², Birgit Strommenger³, Alexander W. Friedrich⁴, and Wolfgang Witte³

¹ Veterinary University Vienna, Department V, Clinic of Surgery and Ophthalmology

² Veterinary University Vienna, Department II, Institute of Bacteriology, Mycology and Hygiene

³ Robert Koch Institute, Wernigerode Branch

⁴ Institute of Hygiene, University Hospital Muenster

Methicillin-resistant *Staphylococcus aureus* (MRSA) from 24 cases of nosocomial infections in a veterinary university hospital occurring sporadically 2003 until 2005 exhibited an uniform pattern of characteristics with regard to molecular typing, antibiotic resistance phenotypes and resistance genes: nearly identical *Sma*I-macrorestriction pattern, MLST ST254 *spa*-sequence type t036, *SCCmec* element of type IVd, resistance to b-lactam antibiotics (*mecA*), oxytetracycline (*tetM*) and Gentamicin (*aph2*"-*aac6*'). MRSA from humans originating from nosocomial infections in Central Europe and also exhibiting MLST ST254 differed from horse MRSA by *spa* sequence type t009 and by containing *SCCmec* elements of types IVa and IVc respectively. MLST ST254 was so far not found among community MRSA in Europe. Therefore a recent transmission of MRSA of ST254 from humans to horses seems unlikely, (Cuny et al. Eurosurveillance 11 (2006) issue 1).

These MRSA were not found to permanently colonise the anterior nares of horses. However, two veterinarians from 43 staff members revealed as nasal carriers. Therefore the possibility of spread to humans cannot be excluded. Of particular interest is one recent purulent infection in a horse by an MRSA of ST398; single isolates of this clonal lineage had also been seen from a dog and from horse in Central Europe.

1L Methicillin-Resistant Staphylococcus Aureus (MRSA) Colonization in Veterinary Professionals

B Hanselman, J Rousseau, S Kruth, JS Weese.

Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1

MRSA is an important cause of hospital-acquired infections in humans worldwide and is increasingly being identified as a community-associated pathogen. The objective of this study was to determine the prevalence of nasal MRSA carriage in veterinary personnel attending an international veterinary conference. Nasal swabs were collected from 417 volunteers at the 2006 ACVIM Forum and processed as previously

described. Isolates were identified as *S. aureus* based on colony morphology, Gram stain appearance, catalase and coagulase reactions and latex agglutination test (LAT). Methicillin-resistance was confirmed via PBP2a LAT and the MIC of oxacillin was determined via Etest. Isolates were typed via *smal* pulsed-field gel electrophoresis (PFGE). Isolates were tested for the presence of the Panton-Valentine leukocidin (PVL) via *lukF-lukS* real time PCR. Risk factors were assessed via stepwise forward logistic regression.

MRSA was isolated from 27/417 (6.5%) volunteers including 22/376 (5.8%) veterinarians, 5/34 (14.7%) technicians and 0/7 others. Large animal practice was the only significant independent risk factor for carriage (OR = 2.9; 95% CI 1.2 – 6.6) with 12/271 (4.4%) small animal personnel, 15/96 (15.6%) large animal personnel and 0/52 others colonized (P<0.001). Two predominant clones were identified using PFGE. Canadian epidemic MRSA-2 (ST5-MRSA-II, similar to USA100) was isolated from 11 small animal and two large animal personnel from the US (n=12) and Denmark (n=1). In contrast, CMRSA-5 (ST8:MRSA:IV, similar to USA 500) was isolated exclusively from large animal personnel (P<0.001) from the US (n=10), UK (n=2) and Germany (n=1). One other isolate, possibly related to CMRSA-2, was recovered from a US small animal veterinarian. No isolates were identified as carrying the genes for PVL production. All MRSA isolates were sensitive to vancomycin and mupirocin.

MRSA carriage may be a significant occupational risk for veterinary personnel and colonized personnel could be sources of infection for patients. Widespread CMRSA-5 carriage in large animal personnel supports previous studies, that suggest this isolate is endemic in the horse population and transmission between horses and humans occurs. In contrast, the predominance of the common CMRSA-2 clone in veterinary personnel in this study, and in household pets in other studies suggests that household pet MRSA is a closer representation of the types of MRSA in the general human population in a given area.

2A The molecular epidemiology of MRSA in the UK

Dr Angela Kearns, Head of Staphylococcus Reference Unit, Centre for Infections, Health Protection Agency, Colindale, London, NW9 5EQ

Historically, the differentiation of strains of *S. aureus* has been reliant on phenotypic tools such as bacteriophage typing, biotyping and susceptibility to antimicrobials e.g. antibiotics, heavy metals and dyes. These techniques often lack reproducibility and provide a low discriminatory index.

The post-genomic era has revolutionised our understanding of the population biology of *S. aureus*. Comparative genomic data from the sequencing of multiple *S. aureus* genomes has helped provide insights into the evolution, antimicrobial

susceptibility transmissibility, virulence and pathogenicity of the species. Careful harnessing of this information and the identification of discriminatory genetic loci affords the opportunity for detailed analysis and fine strain discrimination.

These advances are fundamental to underpinning the epidemiology and surveillance of MRSA in the UK and globally. To sub-type *S. aureus* and explore inter-strain relationships, genomic data has been exploited in a variety of ways. In addition to the more established whole genome methods (e.g. PFGE and PCR- ribotyping), a range of PCR-based methods (e.g. SCCmec, toxin gene profiling and *agr* typing), DNA sequence-based techniques (e.g. MLST and protein A [spa] typing), and DNA chip technology have been used. Collectively, these approaches are complementary and provide powerful tools for epidemiological monitoring.

Over the last two decades, multiple strains of MRSA have been identified in UK healthcare settings, and these are referred to as healthcare-associated MRSA (HA-MRSA). This so-called epidemic MRSA-1 (EMRSA-1) was initially reported causing outbreaks in and around London and became increasingly widespread in UK hospitals in the early 1980s. The emergence of a different strain (EMRSA-2) in the late 1980s prompted a national survey in which a further 12 strains were identified occurring in multiple hospitals and these were referred to as EMRSA-3 to -14. The early 1990s saw the emergence of more successful epidemic strains EMRSA-15 and -16. In the late 1990s, a further three epidemic MRSA strains (EMRSA-17 and Irish-1 and -2) were described. Many of the early epidemic strains have been displaced and, more than 10 years following their emergence, two major clones predominate in healthcare settings in the UK: EMRSA-15 (ST22-MRSA-SCCmecIV) and -16 (ST36-MRSA-SCCmecII). It is noteworthy that the UK epidemic strains were characterised by bacteriophage typing; the application of more robust and discriminatory genotypic methods has shown that not all of the early UK MRSA were unique strain types.

More recently, community-associated MRSA (CA-MRSA) have emerged world-wide. These appear to have evolved in the community and represent a completely distinct entity from HA-MRSA. CA-MRSA have occurred in the UK from about 1998 and represent <2% of the isolates referred to the Reference laboratory for characterisation. They have often been recovered from previously healthy individuals in the community without the classical risk factors for MRSA infection and, primarily, have been associated with sporadic disease ranging from superficial cutaneous infections to fatal necrotising pneumonia. To date, seven different clonal lineages of CA-MRSA have been identified in England and Wales. These observations suggest a new pattern of staphylococcal disease is emerging, presenting a new public health challenge.

The polyclonal nature of HA- and CA-MRSA in the UK emphasises the need to use detailed molecular analyses to help elucidate factors which may be responsible for the

success, survival and dissemination of different clones in healthcare and community settings. Continued vigilance is imperative to underpin epidemiological investigations, help identify risk factors and design effective prevention and control strategies to limit their spread.

2B Evolution: MLST and MRSA

Mark C. Enright, Imperial College London

Multilocus sequence typing (MLST) characterizes variation in core metabolic genes of bacteria allowing analysis of bacterial population structures. The method is invaluable in the study of the epidemiology of pathogenic bacteria and is used worldwide in surveillance of many pathogens. The *Staphylococcus aureus* MLST scheme was developed in 2000 and to date the details of >1500 isolates from 28 different countries have been deposited at the *S. aureus* MLST website <http://saureus.mlst.net/>.

MLST, together with analysis of the Staphylococcal Chromosomal Cassette mec (SCCmec) that carries the methicillin resistance gene, is now widely used to unambiguously define clones of MRSA and the global spread of these important causes of disease can now be tracked. Although the great majority of epidemiological investigations examining *S. aureus* infections have been in humans a number of animal studies have been published. The largest of these studies examined bovine mastitis isolates, the majority of which were found to be unrelated to human isolates from disease and carriage. Preliminary MLST analysis of MRSA and MSSA from equine sources indicate that again distinct lineages are carried in these animals whereas a study of companion animals showed a surprisingly high rate of carriage of the two main causes of MRSA disease in the UK – EMRSA (epidemic MRSA clones) -15 and -16 and there are reports of these clones causing serious invasive disease in these species.

2C Typing of MRSA, the future: VNTR analysis

Prof Peter Hawkey

2D spa typing of methicillin-resistant Staphylococcus aureus isolated from animals and veterinary staff

Arshnee Moodley¹, Funda Bagcigil¹, Marc Stegger², Robert Skov² and Luca Guardabassi^{1*}

¹Department of Veterinary Pathobiology, The Royal Veterinary and Agricultural University, Frederiksberg C, and ²Statens

Serum Institut, Copenhagen, Denmark.

*Presenting author

DNA sequence analysis of the protein A gene variable region (spa typing) provides a rapid and accurate method to discriminate *Staphylococcus aureus* outbreak isolates (1). Such variable region contains 24-bp tandem repeat units, which vary in sequence, number and organization. By spa typing, repeats are assigned a numerical code and the spa-type is deduced from the order of specific repeats. In this study, 22 human, 27 canine, 9 equine, 6 feline, and 3 environmental methicillin-resistant *Staphylococcus aureus* (MRSA) originating from veterinary hospitals in the UK (2-3) and in Ireland (4) were analysed by spa typing and pulsed field gel electrophoresis (PFGE). The aim of the study was to investigate whether spa types correlated with epidemiological information and provided additional information on MRSA transmission between animals and veterinary staff.

All feline and most canine (96%) and human (82%) isolates showed PFGE profiles that were either indistinguishable or closely related to the epidemic clone predominant in the UK (EMRSA-15, clonal complex 22), whereas the vast majority of equine isolates (88%) were related to clonal complex 8. A total of 15 spa types were detected, including 2 novel types (t1041 and t1042). Distinct spa types were detected among strains related to clonal complex 22. The most frequent spa type, t032, was detected in animal and human strains from all veterinary hospitals, whereas the remaining spa types were only detected in strains originating from one hospital. In many cases, the same spa type was found in strains isolated from epidemiologically-related individuals, such as veterinarians and dogs in contact or veterinary staff members within the same clinic. The results were compatible with the notion that MRSA can be transmitted between animals and veterinary staff. It was concluded that spa polymorphism is capable of discriminating between MRSA strains assigned to the same clonal complex by PFGE and can be usefully employed for fast and accurate epidemiological investigation on MRSA carriage in animals and veterinary staff.

References

1. Shopsin, B, Gomez M, Montgomery SO et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* 1999; **37**: 3556-3563.
2. Loeffler A, Boag AK, Sung J et al. Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. *J Antimicrob Chemother* 2005; **56**: 692-7.
3. Baptiste KE, Williams K, Williams NJ et al. Methicillin-resistant staphylococci in companion animals. *Emerg Infect Dis* 2005; **11**: 1942-44.

4. O'Mahony R, Abbott Y, Leonard FC et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. *Vet Microbiol.* 2005; **109**: 285-296.

2E MRSA and microarrays

Dr Jodi Lindsay
St George's, University of London

Microarrays are glass slides with thousands of tiny individual spots of DNA. They are used for two types of studies. Firstly to investigate when genes are expressed under certain conditions or in mutant backgrounds, and secondly to identify genes that are present or missing in different strains (comparative genomics). We have designed and built a *Staphylococcus aureus* microarray that includes polymerase chain reaction (PCR) product spots representing every predicted gene in the first seven *S. aureus* whole genome sequencing projects. This is the most comprehensive *S. aureus* microarray, and one of the most comprehensive bacterial microarrays in the world.

In a comparative genomics study investigating 161 human carriage and invasive isolates of *S. aureus*, we were able to determine that the *S. aureus* genome consists of three parts. 1. Core genes that are found in all strains of *S. aureus* and include many virulence factors. 2. Core variable (CV) genes vary between strains and can be used to classify strains into the dominant lineages matching those determined by MLST. The high number and varied distribution of CV genes indicate that the lineages have evolved relatively independently over a long period of time. CV genes include many surface proteins and their regulators and are predicted to interact with host. 3. Mobile genetic elements (MGE) are discrete pieces of DNA that often integrate into the chromosome, such as plasmids, bacteriophages, pathogenicity islands, staphylococcal cassette chromosomes (SCC) and transposons. MGE encode many virulence and resistance genes and can spread horizontally to new strains. The spread of MGE has led to the emergence of new strains creating novel clinical problems such as epidemic methicillin-resistant *S. aureus* (MRSA), community-acquired MRSA (CA-MRSA) that carry Panton-Valentine leukocidin, and vancomycin-resistant *S. aureus* (VRSA).

The seven strain *S. aureus* microarray is currently being used to identify differences between *S. aureus* strains from different populations, including human carriage and disease in the community and hospital settings, and isolates from bovine, equine, pet and food sources. What does this mean for veterinary medicine? Microarrays are the most comprehensive typing method for determining if two isolates of MRSA are related, which is useful in determining sources of infection or outbreaks. Studies underway may also identify markers of "human" versus "animal" strains, or "virulent" versus "carriage" strains (if they exist).

Microarray studies are also clarifying how *S. aureus* strains are evolving, particularly the acquisition of virulence and resistance genes on MGE.

2F The Molecular Epidemiology of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) in Animals

Williams NJ¹, Dawson S¹, Clegg PD¹, Baptiste KE² and Hart CA³.

¹Department of Veterinary Pathology, University of Liverpool, Neston, Wirral, CH64 7TE, ²The Royal Agricultural and Veterinary College, Denmark, ³Department of Medical Microbiology, University of Liverpool.

Molecular typing of MRSA isolated from animals has gone some way to reveal possible sources of MRSA and the epidemiology of this zoonosis. Differences in epidemic strains in different animal species, may be a reflection of their level of contact with man, as there are differences in the strains found in small animals versus horses.

In the UK previous studies in small animals have largely found isolates identical or closely related to EMRSA-15 (Loeffler et al, 2005) the predominant epidemic health-care associated MRSA strain currently in the UK. In Ireland MRSA from non-equine companion animals were all found to be closely related to each other and the main epidemic human strain, which is similar to the UK EMRSA-15 (O'Mahony et al, 2005). Confirmation of isolates as EMRSA-15 has also been confirmed by multi-locus sequence typing (MLST), with canine MRSA being identified as sequence type (ST) (Waller, 2005). EMRSA-16 (ST36) has also been isolated from dogs in the UK, but to a lesser extent (Rich et al, 2005, Waller, 2006) German MRSA isolates from small animals were also found to be ST22 by multi-locus sequence typing (MLST), with SCCmecIV and were *spa* gene type t032 and negative for panton-valentine leukocidin (PVL) (Strommenger et al, 2006).

In contrast, the situation in the USA and Canada is somewhat different in small animals, with the predominant community-associated Canadian epidemic MRSA-2 (USA100) being found in animals with clinical infections and household contacts (Weese et al, 2006). The latter strains of MRSA were negative for the PVL. More recently CMRSA-10 (USA-300) is being found in small animals in both Canada and the USA, with these isolates being positive for PVL (Weese personal communication), along with other reports of PVL positive MRSA isolates from companion animals (Rankin et al, 2005).

The situation appears to be different in horses, with studies on horses attending a veterinary hospital in Austria with nasal carriage and clinical infections with MRSA demonstrated that isolates were identical or closely related to each other with MLST identifying strains as being ST254. However the isolates were different from human EMRSA 10 strains by PFGE and furthermore the equine isolates exhibited a different SCCmec

cassette type, IVd, compared to IVc in human isolates (Cuny et al, 2006). Irish equine isolates were found to be unique when compared by PFGE to small animal isolates and the predominant Irish human strains, but were all closely related to each other except for one isolate (O'Mahoney et al, 2005). Work in North America has demonstrated the presence of one strain, Canadian MRSA-1 (USA500, ST8, SCCmecIV), which has dominated MRSA isolated from equine clinical cases, from horses in the community and has been found in equine veterinary staff (Weese personal communication), but this strain only accounts for 5% of MRSA infections in Canada. Furthermore ST8 has also been found in the UK and Ireland in horses (Weese personal communication, Waller et al, 2005). In Japan PFGE analysis found equine isolates to be identical or closely related to each other differed from the predominant human MRSA strains in Japan (Shimuzu et al, 1997).

Research undertaken at the University of Liverpool's small animal hospital (SAH) and Philip Leverhulme Equine Hospital (PLEH) have revealed differences in the molecular epidemiology of MRSA in dogs compared to horses. In the SAH, strains identical to EMRSA-15 by macro-restriction PFGE were isolated from three canine clinical cases, as well as three members of staff associated with those cases (Baptiste et al, 2005). These strains were also identified by MLST as ST22 (in collaboration with Mark Enright and colleagues, Imperial College) and were SCCmec IV and *pvl* negative. Isolates from three clinical cases and two staff were all found to be *spa* type t032, however the further member of staff who presented a swab a month later for MRSA isolation had a different *spa* gene type, t020 (Moodley et al, submitted). Further clinical cases from the SAH all in dogs have been identical or closely related to EMRSA-15 by PFGE, of which isolates have been *spa* gene type t032, t1021 and t1041, were all SCCmec IV and negative for the *pvl* gene.

Following two clinical cases involving MRSA in three months in a private small animal veterinary hospital, a cross-sectional study was undertaken to investigate the prevalence of MRSA nasal carriage and animals attending the practice, along with environmental samples. Two of 26 veterinary personnel tested were positive for MRSA, both of which were veterinary surgeons. Eighteen animals attending the surgery were also sampled, however the only animal positive for MRSA was a dog, which belonged to one of the clinicians, this clinician (the owner) was also positive for MRSA. Air samples were taken from all consultation rooms, kennels, preparation room and the operating theatre, with the kennels providing a positive MRSA sample. Environmental surfaces such as examination tables, weighing scales, door handles and hand-wash facilities were sampled. The most common environmental surface, which was positive for MRSA isolation was weighing scales, with examination tables also positive. All isolates were found to be identical by PFGE, were SCCmecIV, PVL negative and were *spa* gene type t020 (CC22), including the isolates which has been from clinical cases some months earlier (in collaboration with Arshnee Moodley, Royal Agricultural College, Denmark).

MRSA has also been isolated from two clinical cases in captive-bred zoo animals, a wallaby presenting with an eye infection and a vulture with a claw abscess, both which were receiving treatment by a clinician who was consistently positive for nasal carriage of MRSA. Animal and human isolates were all identical by PFGE, were SCCmecIV, PVL negative and were all found to be t1043 (CC22) a new *spa* gene type (in collaboration with Arshnee Moodley). MRSA isolates obtained from Bristol University small animal hospital were found to be identical or closely related to EMRSA-15 by PFGE, were also SCCmecIV and PVL negative.

Work undertaken at the PLEH revealed nasal carriage in horses not clinically infected by MRSA, as well as in clinical cases involving MRSA, however, MRSA could not be isolated from veterinary staff at the PLEH (Baptiste et al, 2005). A cluster of equine MRSA isolates in 2004 were all found to be closely related to each other by PFGE and were found to belong to ST254 (EMRSA-10, SCCmec IV) by MLST (in collaboration with Mark Enright), and were *spa* gene type t036, with the exception of one isolate that was ST660, differing by one allele from ST254. The latter sequence types (EMRSA-10) are rarely encountered in the UK. A further equine MRSA was found to be a new sequence type, ST 658 and was SCCmecII positive. Further equine MRSA were found to belong to *spa* gene type t216 (ST-59/CC59) and t127 (CC1), both SCCmecIV. More recently an equine MRSA was found to be identical by PFGE to EMRSA-15 and found to be *spa* type t020 (Moodley et al, submitted).

Also of interest is the prevalence of methicillin-resistant coagulase negative staphylococci (MR-CNS), as they may represent a reservoir of SCCmec for methicillin-sensitive *S. aureus* (MSSA), as well as being multi-drug resistant and known to cause infections in animals. Work at Liverpool has found large difference between MR-CNS in small animals compared to horses. One survey of dogs and cats at the SAH found MR-CNS were rarely isolated, however surveys at the PLEH and other large equine hospitals found a high prevalence of MR-CNS (Baptiste et al, 2005, Meehan, MSc Thesis, 2005). Largely the equine MR-CNS although *mecA* positive were found to contain SCCmec cassettes, which did not correspond to types I-IV; except in three cases, types I, II and IV were found. Furthermore a *S. scuri* isolate positive for SCCmecIV was also positive for the *femA* gene of *S. aureus* (Meehan, MSc Thesis, 2005). More recently MR-CNS has been isolated from both canine clinical cases at the SAH and MR-*S. epidermidis* amongst other MR-CNS have also been isolated from equine clinical cases at the PLEH and have been found to carry SCCmec IV.

It is clear that there are differences in the epidemiology of MRSA found in small animals versus horses. MRSA appears to be more common in small animals with strains reflecting those most prevalent within the human population and given the contact between small animals and their owners, accompanied by frequent visits to veterinary clinics, there is much greater potential for transmission between small animals and man.

In contrast, MRSA infections and carriage appears to occur at lower frequency in horses and the strains found in horses are distinct from the major epidemic strains in the human population and may be adapted to colonisation of the horse. One marked difference between equine and small animal strains of MRSA from work done at the University of Liverpool is their antimicrobial susceptibility, with equine strains largely being resistant to a wider range of antibiotics, this was also observed in Ireland (O'Mahony *et al*, 2005). All equine strains were gentamicin resistant, an antibiotic commonly used in equine medicine and this phenotype may have contributed to the selection of such strains. The majority of animal MRSA have carried SCCmecIV the smallest of the SCCmec cassettes. The higher apparent prevalence of MRSA in small animals in the UK, compared to horses may be due to the fact that in addition to methicillin, small animal MRSA are largely resistant to ciprofloxacin, but few other antibiotics. It is known that antibiotic resistance confers a cost to organisms and in the absence of antibiotics, resistant organisms do not compete as well with sensitive strains. However, small animal MRSA having a smaller SCCmec cassette and fluoroquinolone resistance, which is usually due to chromosomal mutations, may be able to compete equally with MSSA, whereas equine MRSA in the absence of antibiotics due to their greater MDR status may not.

References

Baptiste KE, Williams K, Williams NJ, Wattret A, Clegg PD, Dawson S, Corkill JE, O'Neill T and Hart CA (2005). Methicillin-resistant staphylococci in companion animals in the North-west of England. *Emerg.Infect.Dis.* 11(12);1942-1944.

Cuny C, Kuehmerle J, Stanek C, Willey B, Strommenger B and Witte W (2006). Emergence of MRSA infections in horses in a veterinary hospital: strain characterisation and comparison with MRSA from humans. *Euro.Surveill.*11(1).

Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, Dalgaard A, Smith H, Stevens K and Lloyd DH (2005). Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. *J.Antimicrob.Chem.*56;692-697.

Meehan L (2005). Survey of Methicillin Resistant *Staphylococcus aureus* and Methicillin Resistant Coagulase Negative Staphylococci in UK Equine Hospitals. VIDC MSC thesis, University of Liverpool.

Moodley A, Stegger M, Bacgcigil1 F, Baptiste K, Loeffler A, Lloyd DH, Williams NJ, Leonard N, Skov R and Guardabassi L. spa typing of methicillin-resistant *Staphylococcus aureus* isolated from domestic animals and veterinary staff in the UK and in Ireland. Submitted to the *Journal of Antimicrobial Chemotherapy*.

O'Mahony R, Abbott Y, Leonard FC, Markey BK, Quinn PJ, Pollack PJ, Fanning S and Rossney AS (2005). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. *Vet.Microbiol.*109;285-296.

Rankin S, Roberts S, O'Shea K, Maloney D, Lorenzo M and Benson CE (2005). Panton valentine leukocidin (PVL) toxin positive MRSA strains from companion animals. *Vet.Microbiol.* 108;145-148.

Rich M, Roberts L and Kearns A (2005). Methicillin-resistant staphylococci isolated from animals. *Vet.Microbiol.* 105;313-4.

Shimuzu A, Kawano J, Yamamoto C, Kakutani O, Anzai T and Kamada M (1997). Genetic analysis of equine methicillin-resistant *Staphylococcus aureus* by pulsed-field gel electrophoresis. *J.Vet.Med.Sci.*59 (10):935-937.

Strommenger B, Kehrenberg C, Kettlitz C, Cuny C, Verspohl J, Witte W and Schwarz S (2006). Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. *J.Antimicrob.Chem.*57;461-465.

Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B and Low DE (2006). Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. *Vet.Microbiol.*In Press.

Waller A (2005). MRSA update. DEFRA/AHT/BEVA.Eq.Quart.Dis.Surveill.Rep. Vol 1(2) April-June.

Weese JS.(2006) Personal communication.

2G Concordance of cluster analyses based on spa-typing, MLST and SmaI-macrorestriction analysis – application to veterinary isolates

B. Strommenger¹, C. Kettlitz¹, B. Pasemann¹, G. Werner¹, J. Rothgänger³, D. Harmsen² und W. Witte¹

¹ Robert Koch Institute, Wernigerode Branch, Wernigerode, Germany

² Department of Periodontology, University Hospital Münster, Münster, Germany

³ Ridom GmbH, Würzburg, Germany

Until now *SmaI*-macrorestriction analysis (PFGE) was regarded the "gold standard" in *S. aureus* strain typing, although it has the disadvantage of insufficient comparability between results obtained from different laboratories. During the past 5 years DNA sequence based typing methods like multilocus sequence typing (MLST) and *spa*-typing became more frequently used because of some obvious advantages, like ease of use, reproducibility, transportability and

comparability of results. However, in contrast to PFGE and MLST data, until now no algorithm was available to group related *spa*-types together for epidemiological investigations. The implementation of BURP into the Ridom StaphType software allows clustering of different *spa*-types based on a new algorithm for the alignment of repeat sequences. Thus, the aim of the present study was to compare clustering results obtained by *spa*-typing/BURP analysis to those obtained by well established methods (*SmaI*-macrorestriction analysis and MLST/eBURST).

A collection of clinical *S. aureus* strains including MRSA and representing major clonal lineages associated with important kinds of infections which have been prevalent in Germany and Central Europe during the last ten years were used for comparison. Although *SmaI*-macrorestriction analysis revealed the highest discriminatory power, clustering results for all three methods were fairly concordant.

Including *spa*-typing data for MRSA of veterinary origin we could show that, although *spa*-types were different from those of human isolates in certain clonal complexes (as defined by MLST), BURP was able to group those *spa*-types together with related types apparent in human medicine.

The results of this study indicate that *spa*-typing, together with BURP clustering, is a useful tool in *S. aureus* epidemiology, especially because of ease of use and the advantages of unambiguous sequence analysis as well as reproducibility and exchange of typing data.

2H Multi Locus sequence typing (MLST) and cassette chromosome mec (SCCmec) characterisation of Methicillin-resistant *Staphylococcus aureus* isolated from animals and veterinary personnel in Ireland.

Rebecca O'Mahony¹ Noelle Brennan², Mary Booth², Yvonne Abbott¹ and Nola Leonard¹

School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Dublin, Ireland¹ and Athlone Institute of Technology, Athlone, Co WestMeath, Ireland².

Reports of methicillin-resistant *Staphylococcus aureus* (MRSA) in animals and veterinary personnel have become more frequent in recent years^{1, 2, 3 and 4}. Application of multilocus sequence typing (MLST) and SCCmec typing are two methods that allow for the characterization of both the strain phylogeny and the evolutionary relationship of MRSA clones. In this study we examine a collection of MRSA isolates from animals and associated veterinary personnel. The collection was obtained from a university veterinary hospital and several private veterinary clinics in different locations throughout Ireland. MRSA was recovered from 21 animals comprising of 11 dogs, 7 horses, 1 rabbit, 1 seal, 1 cat and 6 attendant veterinary personnel.

Multi locus sequence typing identified two sequence types (ST) within the collection. MRSA isolated from seven horses and their attendant personnel (3) had the same sequence type, ST8. This strain has not been reported previously in Irish human patients whilst it has been reported worldwide. Sequence type ST 22 was found in all canine (11), feline (1), porcine (1), lapine (1) and human (3) MRSA isolates. ST22 is the most common strain type found in human hospitals in Ireland. Preliminary SCCmec typing using a single plex PCR assay indicated that type IV or V were absent in all MRSA isolates. A similar situation has recently been reported where the lack of amplicons for the class B gene complex associated with type IV allotypes was observed⁵. Further SCCmec characterisation will provide information on whether a new variant (NV) of type IV may be present in the collection and will also confirm if the isolates are community acquired (CA) or hospital acquired (HA). Finally, this study shows transmission of two strains of MRSA is occurring in veterinary practices in Ireland. It also suggests transmission between animals and humans can be of both a veterinary and public health concern.

[1] Cuny, C., Kuehmerle, J., Stanek, C., Willey, B., Strommenger, B. and Witte, W. Emergence of MRSA infections in horses in a veterinary hospital: strain characterization and comparison with MRSA from humans. 2006. Eurosurveillance monthly release.

[2] O' Mahony, R., Abbott, A., Leonard, F.C., Markey, B., P.J. Quinn., P.J. Pollock., Fanning, S. and Rossney, A. 2005. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. *Veterinary Microbiology.* 109 285-296.

[3] Baptiste, K., Williams, K., Williams, N., Wattret, A., Clegg, P., Dawson, S., Corkill, J., O' Neill, T., and Hart, C. 2005. Methicillin-resistant staphylococci in companion animals. *Emerg Infect Dis.* 12 1942-1944

[4] Weese, J., Dick, H., Willey, B., McGeer, A., Kreiswirth, B., Innis, B. and Low, D. 2006. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. *Vet Microbiol.* Epub ahead of print.

[5] Sola, C., Cortes, P., Saka, H., Vindel, A. and Bocco, J. 2006. Evolution and molecular characterization of methicillin-resistant *Staphylococcus aureus* Epidemic and sporadic clones in Cordoba, Argentina. *J Clin Micro.* 44 192-200.

2J Rapid, reliable sub-typing of methicillin resistant *Staphylococcus aureus* by denaturing high pressure liquid chromatography and DNA sequence analysis

F. Jury¹, M. Al-Mahrous², A. Fox³, W. Ollier¹ & M. Upton²

¹Centre for Integrated Genomic Medical Research, University of Manchester, Stopford Building, Oxford Road, Manchester, M13 9PT, UK. ²Department of Medical Microbiology, University of Manchester, Clinical Sciences Building, Manchester Royal Infirmary, Oxford Road, Manchester, M13 9WL, UK. ³Health Protection Agency North West Laboratory, Manchester Medical Microbiology Partnership, Clinical Sciences Building, Manchester Royal Infirmary, Oxford Road, Manchester, M13 9WL, UK.

Objectives

Methicillin resistant *Staphylococcus aureus* (MRSA) is a significant cause of hospital- and community-acquired disease. Epidemic MRSA (EMRSA) strains are particularly successful pathogens able to spread rapidly. Detection and sub-typing of EMRSA can require a week of investigation. To be of true use, methods for identification of cross-infection events and outbreaks need to be highly discriminatory and easy to perform. The current methods for this purpose have numerous disadvantages including expense and a lack of standardisation and rapidity. Characterisation of the X region of the *spaA* gene provides a rapid, unambiguous, discriminatory sub-typing method that can inform outbreak investigation. Denaturing high-performance liquid chromatography (DHPLC) is a powerful technique for separation and quantitation of nucleic acids. We have used DHPLC to detect DNA sequence variation within the *spaA* gene to fully investigate the application of DHPLC approaches as a possible rapid and cheap alternative to *spa* typing by conventional methods.

Methods

Genomic DNA was extracted from cultured isolates and used as template in PCR to amplify the X region of the *spaA* gene. DHPLC was carried out by Transgenomic WAVE™ DNA fragment analysis. Retention times were collected for each sample and comparisons to a size standard were made and the number of repeat units for each sample was calculated. Sequence data were also obtained for each of the samples. Samples were then subjected to heteroduplex analysis against a reference sample to identify those samples with different repeat organisations, in order to identify any local evolution or introduction to the local area of new *spa* types.

Results

Sequence data indicated a predominance of strains with 15 (n=13; group A) or 16 (n=8; group B) repeat units in the *spaA* X region. The number of repeat units in each sample matched exactly those predicted by WAVE™ analysis. Heteroduplex analysis was able to distinguish those samples that contained a different repeat organisation to that of the

reference sample, and this was also confirmed by sequence analysis.

Conclusion

DHPLC methods can rapidly detect repeat unit number with a very high correlation to sequence data. This indicates the ability of the WAVE™ to rapidly sub-type strains of MRSA, which differ by a single repeat unit. Further sub-typing to indicate local evolution of the *spaA* X region, is possible through heteroduplex analysis.

Poster Abstracts

P1 Validation of a Real-Time Polymerase Chain Reaction Assay for Rapid Identification of Methicillin-Resistant *Staphylococcus Aureus* Directly from Nasal Swabs in Horses

MEC Anderson, JS Weese, Ontario Veterinary College, Guelph, ON, Canada, N1G 2W1

Outbreaks of methicillin-resistant *Staphylococcus aureus* (MRSA) infection and carriage in horses have recently been reported in numerous locations worldwide. Zoonotic transmission and human-to-horse transmission of MRSA have been documented. One of the major obstacles to control of equine MRSA is timely identification of infected and colonized horses. Typically identification of MRSA from clinical specimens or screening swabs takes from 3-7 days. Molecular identification techniques such as real-time polymerase chain reaction (RT-PCR) have recently been employed in human medicine to reduce this turn-around time to as little as 1-2 hours. The purpose of this study was to compare a RT-PCR assay for rapid identification of MRSA directly from nasal swabs in horses to standard culture techniques.

Nasal swabs collected from 294 clinically normal horses from Ontario and Kentucky were processed using a commercial RT-PCR assay previously validated for use with human specimens (IDI-MRSA, GeneOhm Sciences, San Diego, CA) according to the manufacturer's directions. Following broth enrichment, the swabs were also cultured on mannitol salt agar containing 2 µg/ml oxacillin, and MRSA colonies were identified using biochemical and latex agglutination tests as per standard protocols.

From the results of the first run of PCR, 2/177 and 168/177 samples were positive and negative, respectively, by both PCR and culture. Seven of 177 samples were positive by PCR and negative by culture, whereas 0/177 samples were negative by PCR and positive by culture. The *kappa* statistic was 0.35, which represents poor agreement between the two tests. Of the remaining 117 samples, on the first run of PCR 12 failed the external control and were excluded, while 105 samples were reported as "unresolved".

Following one freeze-thaw cycle of the lysates, the recommended technique to resolve such samples, 61/110 (55%) samples remained unresolved, which represents 21% of all samples tested.

In this study, the IDI-MRSA assay was not a clinically practical screening test for horses harbouring MRSA in the nose. Its agreement with culture, the current accepted standard, was poor, although the lower than anticipated sample prevalence of MRSA colonization may have impacted the results. Regardless, the unresolved rate was also very high (37%) on initial testing of samples, which significantly decreases the clinical utility of the test. The reason for the high unresolved rate compared to that found with human specimens is unknown. Modification of the technique for sample processing may help decrease the unresolved rate, but this requires further investigation.

P2 Screening of horses and dogs in Slovenia for carriage of methicillin-resistant staphylococci

M. Vengust¹, V. Cestnik¹, M.E.C. Anderson², J.S. Weese²

¹University of Ljubljana, Veterinary Faculty, Slovenia, SI-1000; ² Department of Clinical Studies, University of Guelph, Ontario, Canada N1G 2W1

Various staphylococci are commensal microflora of domestic animals yet can also be opportunistic pathogens. Coagulase positive staphylococci such as *S. aureus*, *S. intermedius* and *S. schlieferi* subsp *coagulans* are important causes of disease in dogs and cats. Methicillin-resistant strains of *S. aureus* (MRSA) are of particular concern and can also be transmitted between animals and humans. MRSA is an important pathogen in humans in Slovenia; yet, MRSA infections in veterinary species have not been reported. The objective of this study was to evaluate the prevalence of methicillin-resistant staphylococcal carriage in clinically normal dogs and horses in Slovenia.

Three hundred clinically normal horses of various breeds from 14 farms were enrolled. Horses selected were housed in publicly open establishments used for tourist/show purposes (n = 100), riding schools and recreational facilities (n = 100), and competition horses (n = 100). One nasal swab was collected from horses.

Two hundred clinically normal dogs of various breeds were enrolled. These consisted of agility competitors (n = 70), rescue/working dogs in training (n = 70), and household pets (n = 60). Two swabs were taken from dogs from: 1) anterior nare, and 2) a combination of perineal area and 0.5 cm into the anus.

Direct culture was performed by inoculating swabs onto mannitol-salt agar with 2 µg/ml oxacillin. Enrichment culture was performed by incubating swabs in broth containing 7.5%

NaCl for 24 hours prior to inoculation onto mannitol-salt agar with 2 µg/ml oxacillin.

MRSA was not isolated from any sample. Methicillin-resistant *S. intermedius* was isolated from 3 (1.5%) dogs; one from the nasal swab only and two from both the nasal and perineal/rectal swabs.

Methicillin-resistant coagulase negative staphylococci were present in 126/300 (42%) horse samples and 26/200 (13%) dogs: 15 from nasal swabs only, 6 from rectal/perineal swabs only and, 2 from both swabs.

It was interesting that MRSA was not identified in any animal in this study, considering the role of this pathogen in disease in Slovenia. Possible reasons for this include absence of MRSA in the animals in the country, a low population prevalence that was not detectable using this sample size, or clustering in animals in groups that were not sampled. Further study of animals in Slovenia is required to detect emergence of this potential veterinary and zoonotic pathogen, and to characterize its epidemiology.

P3 Occurrence and species distribution of mecA-positive coagulase-negative staphylococci in the nasal cavity of animals in Denmark

Arshnee Moodley¹, Funda Bacgicgil¹, Keith Edward Baptiste² and Luca Guardabassi¹

¹Department of Veterinary Pathobiology and ²Department of Large Animal Sciences, The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark

Coagulase-negative staphylococci (CNS) are the predominant staphylococci in the resident flora of healthy skin in animals (1). CNS isolated from animals such as *S. haemolyticus*, *S. sciuri*, *S. lentus*, *S. saprophyticus*, *S. xylosus* and *S. epidermidis* are known to carry *mecA* (2-3), the gene encoding methicillin resistance in *S. aureus*. Recent studies have shown that methicillin-resistant *Staphylococcus aureus* (MRSA) can be isolated from the nasal cavity of dogs, horses and pigs (1-3). The aim of the present study was to determine frequency of isolation and species distribution of methicillin-resistant staphylococci in the nasal mucosa of dogs, horses, swine and cattle in Denmark.

Nasal swabs were collected from 100 animals of each species. Canine and equine samples were obtained from animals attending the small animal and large animal hospitals at The Royal Veterinary and Agricultural University and a large horse facility. Samples from swine and cattle were collected at slaughterhouses immediately after killing. Methicillin-resistant staphylococci were isolated by selective enrichment followed by plating on oxacillin resistance screening agar base and on 5% blood agar with 30µg cefoxitin disks.

Following *mecA* detection by PCR, *mecA*-positive isolates were typed by random amplified polymorphic DNA (RAPD) analysis and one strain representative of each RAPD profile was identified by 16S rDNA sequencing.

No MRSA were detected among the 400 animals tested. The isolation frequencies of *mecA*-positive CNS in horses and dogs were 51% and 13%, respectively. The species isolated from horses were *S. haemolyticus* (n=19), *S. vitulinus* (n=19), *S. sciuri* (n=12), and *S. epidermidis* (n=1). Among the canine isolates, 7 isolates were identified as *S. epidermidis*, 3 as *S. haemolyticus*, 2 as *S. warneri* and 1 as *S. sciuri*. To the best of our knowledge, this is the first time that *mecA* is reported in *S. vitulinus* and *S. warneri*. The results indicate that methicillin-resistant CNS are relatively more common in horses and dogs than in food animals. Accordingly, CNS represent a potential reservoir for acquisition of *mecA* by *S. aureus* in these animal species.

References

1. Nagase N, Sasaki A, Yamashita K, et al. Isolation and species distribution of staphylococci from animal and human skin. *J Vet Med Sci* 2002; **64**: 245-250.
2. Yasuda R, Kawano J, Onda H et al. Methicillin-resistant coagulase-negative staphylococci isolated from healthy horses in Japan. *Am J Vet Res* 200; **61**: 1451-1455.
3. Kawano J, Shimizu A, Saitoh Y et al. Isolation of methicillin-resistant coagulase-negative staphylococci from chickens. *J Clin Microbiol* 1996; **34**: 2072-2074.
4. Loeffler A, Boag AK, Sung J et al. Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. *J Antimicrob Chemother* 2005; **56**: 692-697.
5. Baptiste KE, Williams K, Williams NJ et al. Methicillin-resistant staphylococci in companion animals. *Emerg Infect Dis* 2005; **11**: 1942-1944.
6. Voss A, Loeffen F, Bakker J, Klaassen C, and Wulf M. Methicillin-resistant staphylococci in pig farming. *Emerg Infect Dis* 2005; **11**: 1965-1966.

P4 Prevalence of Methicillin-Resistant *Staphylococcus Aureus* Carriage in Dogs Entering a Veterinary Teaching Hospital

B. Hanselman, M. Anderson, J. Rousseau, S. Kruth, and J.S. Weese.

Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of hospital-acquired infections in human

hospitals worldwide and is increasingly being identified as a community-associated pathogen. The objective of this study was to determine the prevalence of MRSA carriage in a canine population that presented to the Veterinary Teaching Hospital, Ontario Veterinary College. A convenience sample of 203 dogs was studied. No dogs had clinical signs suggestive of bacterial infection. Cotton swabs were used to collect nasal, axillary and rectal samples from each dog. Swabs were plated directly onto mannitol-salt agar with 2 µg/ml oxacillin and incubated at 35°C for 48 hours. Swabs were also enriched in broth prior to culture. Isolates were identified as *S. aureus* based on colony morphology, Gram stain appearance, catalase and coagulase reactions and latex agglutination test (LAT). Methicillin-resistance was confirmed via PBP2a LAT and the MIC of oxacillin was determined via Etest.

MRSA was isolated from the nasal passages, but not rectum or axilla, of 2/203 (1%) dogs. In addition, 2 *S. aureus* isolates were cultured from 2 other dogs (nasal, axillary sites) that possessed a borderline-resistant MRSA associated with high-level β-lactamase production. Methicillin-resistant *S. intermedius* (n=2) and *S. schleiferi* (n=1) were isolated from rectal and nasal sites of other dogs. Clinical infections were not present in any dogs carrying methicillin-resistant staphylococci. Carriage with MRSA was identified in a small percentage of this population of dogs. Further study is required to evaluate risk factors for MRSA colonization and implications of MRSA colonization of dogs for other animals, owners and veterinary personnel.

P5 Antimicrobial Susceptibility of Oxacillin-Resistant Staphylococci from Dogs

A. Hillier¹, S.C. Rankin²

¹College of Veterinary Medicine, The Ohio State University, 601 Vernon L. Tharp Street, Columbus, OH, USA

²Matthew J Ryan Veterinary Hospital of the University of Pennsylvania, 3900 Delancey Street, Philadelphia, PA, USA

Staphylococcus species isolated from the skin of dogs include *S. intermedius*, *S. schleiferi*, *S. aureus* and coagulase-negative staphylococci. Recent reports indicate the emergence of oxacillin-resistant staphylococci from dogs with bacterial skin infections. By convention, staphylococci that are resistant to oxacillin by in vitro susceptibility tests are reported by clinical microbiology laboratories as resistant to all β-lactam antibiotics. These antibiotics are therefore not recommended for treatment of oxacillin-resistant staphylococcal infections. The purpose of this study was to investigate the susceptibility of oxacillin-resistant staphylococci isolated from skin lesions and carriage sites of dogs with superficial bacterial folliculitis. Organisms were collected with a sterile culturette from carriage sites (nasal mucosa, anal mucosa or nonaffected axillary skin) and skin lesions and cultured. Isolates were speciated using the MicroScan Walkaway 40 system (Dade Behring,

Sacramento, CA) and susceptibility testing was performed by Kirby-Bauer (KB) disk diffusion and by broth microdilution (MicroScan). The presence of PBP2a was detected using a commercial latex agglutination test (Oxoid PBP 2' Test, Remel, Lenexa, KS) and the *mecA* gene was detected via PCR. Ten isolates from 6 dogs were studied, including 4 isolates from skin lesions of 4 dogs and 6 isolates from carriage sites of 3 dogs. Isolates included *S. intermedius* (2), *S. schleiferi subsp. coagulans* (4), *S. aureus* (1), *S. epidermidis* (2) and *S. xylosus* (1). All 10 isolates carried the *mecA* gene and were positive for PBP2a. Susceptibility or intermediate susceptibility to the following β-lactam antibiotics were recorded by KB/Microscan respectively: oxacillin (1/1), cephalothin (10/not done [ND]), cefazolin (ND/9), cefpodoxime (6/ND), amoxicillin/clavulanic acid (8/7), imipenem (ND/8). All isolates were resistant to oxacillin on the oxacillin salt agar screen test. All isolates were susceptible to chloramphenicol and vancomycin, with variable susceptibility or resistance to other antibiotics. One of the dogs infected with oxacillin-resistant *S. intermedius* was treated with cephalexin (25 mg/kg q 12 hours for 4 weeks) resulting in complete resolution of the lesions. The results of this study suggest that the convention of reporting all oxacillin-resistant staphylococci as resistant to all β-lactam antibiotics may not be applicable for all staphylococci isolated from the skin of dogs. Antimicrobial susceptibility and treatment of animals infected with oxacillin-resistant staphylococci should be evaluated in a larger controlled study.

P6 Frequency of Methicillin-Resistant *Staphylococcus Aureus* Isolated from Dogs Visiting Hospitalized People in Ontario

S. Lefebvre¹, J. S. Weese², D. Waltner-Toews¹, R. Reid-Smith¹.

Depts. of Population Medicine¹ and Clinical Studies², University of Guelph, Ontario, Canada.

Background/Objectives

Approximately 90% of Ontario hospitals permit dogs to visit their patients (Lefebvre *et al*, in press). Few of these hospitals enforce infection control protocols (if any) for patient-dog interactions, providing opportunities for zoonotic transmission of pathogens. We sought to determine the prevalence and incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) in a subset of visitation dogs.

Methods

Prevalence was determined through the collection of nasal swabs and fecal specimens from a group of 102 healthy dogs actively involved in visitation programs in Ontario during the summer of 2004 (Lefebvre *et al*, in press). Incidence rates are currently being measured through a prospective cohort study that began in May, 2005 and involves 100 newly inducted hospital visitation dogs and 100 dogs without exposure to healthcare facilities, recruited from therapy dog

organizations in Ontario and Alberta. For the latter study, fecal specimens and nasal swabs are being submitted by owners once every 2 months for one year, the last of which are due in October, 2006. Along with each submission, owners are required to forward a log detailing any antimicrobial use within the home during the period under study. All specimens submitted to date have been cultured for MRSA using enrichment techniques and the identities of any isolates confirmed via standard laboratory testing.

Results

No MRSA was isolated from any of the specimens collected during the prevalence study. All of the specimens collected at the beginning of the incidence study also tested negative for MRSA. However, since that time and up until January 31, 2006, MRSA has been isolated from the feces (but not the associated nasal swabs) of 2 visitation dogs and 1 dog from the unexposed cohort for estimated incidence rates of 0.06/dog-year and 0.03/dog-year respectively. According to the log books received, there was no evidence of antibiotic use within the home for any of these animals at or preceding the time that the specimens tested positive. We have been unable to isolate MRSA from any of these dogs since, suggesting a maximum duration of shedding of 2-4 months for each.

Conclusions

More information is required before we can conclude whether exposure to human healthcare facilities is a risk factor for dogs acquiring MRSA. Community-associated strains of MRSA are also emerging, and need to be considered as potential sources in addition to hospital-associated strains. For the time being, we recommend that all dogs be prevented from visiting with MRSA-positive patients, and that MRSA-positive dogs be barred from visiting any patient

P7 Multilocus sequence typing of coagulase positive staphylococci causing disease in animals

Mairi Mitchell¹, Amanda Boag², Tim Parkin¹, Steve Shaw¹, Jaana Pauls¹, Grant Gooch¹ and Andrew Waller¹

¹Animal Health Trust, Newmarket, England. ²Royal Veterinary College, Hawkshead, England.

There is increasing evidence that MRSA is no longer restricted to humans and is now emerging as an important cause of zoonotic and veterinary disease. We hypothesize that MSSA and MRSA strains, which infect animals have been acquired following transmission from humans.

To test this hypothesis we are using multilocus sequence typing (MLST) (Enright *et al.*, 2000) to determine the identity of 100 different strains of *S. aureus* (50 MSSA and 50 MRSA), isolated from swabs taken from animals throughout the UK and sent to the AHT and RVC diagnostic laboratories.

We have determined the identity of 26 strains of MRSA isolated from clinical cases in dogs, 2 from horses and 1 from a cat. Our results suggest that all MRSA strains identified to date are identical or closely related to those commonly identified in humans. Twenty four of the 26 canine MRSA isolates were EMRSA-15, one was a novel single locus variant of EMRSA-15 and one was an EMRSA-16 sequence type. One of the equine MRSA strains was EMRSA-16 and the other was ST8 (EMRSA 2, 6, 7, 12, 13 or 14). The feline MRSA isolate was EMRSA-15.

Canine MSSA strains were also related to human *S. aureus* sequence types highlighting the close contact shared between humans and companion animals. In contrast equine MSSA strains were generally distinct from human sequence types and more closely resembled those previously identified in bovines and caprines.

S. aureus is responsible for only 10% of coagulase positive staphylococcal infections in animals, the majority being caused by *S. intermedius*. Recently, we identified our first case of methicillin resistant *S. intermedius* in a dog. In order to monitor the spread of antibiotic resistance in *S. intermedius* we aim to develop a new MLST scheme based on that used for *S. aureus*. In a pilot study, forty-two strains have been sub-typed based on the sequence of a 450bp internal fragment of the *pta* gene. Initial phylogenetic analysis indicates that canine isolates are closely related to one another, but are distinct from those infecting horses. These data will also enable the identification of representative *S. intermedius* strains for the study of host specificity and for the design of effective vaccines.

References

Enright MC, *et al.*, 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol.* 2000 38:1008-15.

P8 Investigating the transfer and viability of Methicillin Resistant *Staphylococcus aureus* (MRSA) in standard vetbed and medibed in vitro

F.L. Ruedisueli

Department of Biological Sciences, University of Lincoln, UK

Methicillin resistant *Staphylococcus aureus* (MRSA) are being isolated from companion animals more frequently in recent times. This poses the potential risk of MRSA transfer from animal to human, but also poses a risk to companion animals themselves. With an increase of human and animal MRSA carriers, the risk of mainly canine MRSA infections in veterinary surgeries is likely to increase.

MRSA can be transmitted via direct contact, aerosols as well as inanimate objects. Hence veterinary practices should also

consider a-biotic factors in hygiene management. As veterinary bedding can become contaminated with MRSA via a canine or human carrier, it could potentially infect post-surgical wounds. To assess the reality of this scenario, this study investigated the viability of MRSA in 2 types of vetbedding in vitro.

MRSA (Oxoid, UK), cultured on BBA, was used to create a bacterial suspension at 2 different concentrations, on which viable counts were performed. Patches (16cm²) of sterile washed vetbed and medibed were inoculated with the MRSA suspension using sterile swabs. The inoculated vetbed and medibed patches (and controls) were left contaminated for 24 and 48 hrs at room temperature after which residual viability was tested via contact plate inoculation.

Inoculation concentrations of all the test patches were 2*10¹⁰CFU and 1*10⁶CFU. Both concentrations resulted in excess growth from vetbed at 24 and 48hrs after contamination, following contact plate inoculation. Controls showed no growth, demonstrating the absence of accidental environmental contamination. No growth was observed from the medibed patches for both concentrations at 24 and 48hrs after contamination, following contact plate inoculation.

The results show that MRSA can remain viable in vetbed for at least 48 hrs. This indicates the potential risk of bedding remaining a source of MRSA infection, long after initial contamination by owner, dog or veterinary practice staff. Results also indicate that this risk can be minimised by using anti-microbial vetbed (medibed). This may offer an additional tool in MRSA prevention in companion animals within veterinary surgeries or the home.

P9 Molecular characterization of methicillin resistant *Staphylococcus aureus* strains derived from pet animals – are they related to human isolates?

B. Strommenger¹, C. Kehrenberg², C. Kettlitz¹, C. Cuny¹, J. Verspohl³, W. Witte¹ and S. Schwarz²

¹ Robert Koch Institute, Wernigerode Branch, D-38855 Wernigerode, Germany

² Institut fuer Tierzucht, Bundesforschungsanstalt fuer Landwirtschaft (FAL), D-31535 Neustadt-Mariensee, Germany

³ Institut fuer Mikrobiologie, Zentrum fuer Infektionsmedizin, Stiftung Tieraerztliche Hochschule Hannover, D-30173 Hannover, Germany

In order to assess the genetic relationship between Methicillin resistant *Staphylococcus aureus* (MRSA) isolates from pet animals and humans a collection of pet isolates was characterized and compared to human isolates from clonal complexes most prevalent in Central Europe.

Methods

S. aureus isolates were investigated for their in vitro susceptibility to antimicrobial agents by broth microdilution. Resistance genes and the Panton-Valentine leucocidin gene *lukF-lukS* were identified by PCR. All isolates were characterized by *Sma*I-macrorestriction analysis and *spa*-typing to assess their genomic relationships. Representative isolates were additionally analyzed by multilocus sequence typing (MLST) and PCR-directed *SCCmec* typing.

Results

All pet isolates carried the resistance genes *mecA* and *erm(C)* and proved to be resistant to β -lactams and MLS_B antibiotics. In addition, all isolates were resistant to fluoroquinolones. None of the pet isolates carried the Panton-Valentine leucocidin gene *lukF-lukS*. Macrorestriction analysis revealed that the pet MRSA isolates exhibited four closely related *Sma*I fragment patterns. Moreover, all isolates revealed *spa*-type t032. Further analysis of representatives of the different PFGE types revealed the presence of multilocus sequence type ST22 in combination with a type IV *SCCmec* element.

Conclusions

Based on their strain characteristics, the MRSA isolates from pets investigated in this study closely resembled ST22 MRSA isolates which are widely disseminated in German hospitals. Results of this study indicate that cross transmission of MRSA between pet animals and humans might have occurred.

P10 An investigation of the usefulness of Oxoid chromogenic media in the rapid differentiation of *Staph.intermedius* from MRSA isolates in dogs

Peter Webb MRCVS Tony Bacon FIMLS Axiom Veterinary Labs, The Manor House, Newton Abbot TQ12 4PB

The recovery of methicillin (oxacillin) resistant *Staphylococcus aureus* (MRSA) from man is well documented and subject to many publications. Recently there have been reports on the recovery of this organism from multiple veterinary species prompting zoonotic concern. However *S.aureus* is rarely recovered from dogs, and recent studies have shown poor adherence of *S. aureus* to canine keratocytes thus *Staph aureus* is not considered a primary pathogen of dogs. It is nevertheless an important opportunist with zoonotic implications rendering its identification in veterinary cultures important. The pathogenic staph. recovered from dogs is *S. intermedius*. Veterinary applications can contain both *S.intermedius* and *S.aureus*; both species have similar cultural characteristics on routine culture making differentiation difficult. Definitive differentiation relies on sugar fermentation (through commercial kits) and even these produce similar patterns with the exception of mannitol and VP. *S.intermedius* is partially mannitol negative and VP negative. This is a time consuming and costly exercise when all coagulase + staph cultures have to be evaluated in this fashion

There are many published cultural methods for the routine recovery of MRSA in man; it is, however recognised no one media recovers every strain. These specific media are less helpful in routine veterinary investigations applications where the media may not be available and/or supports growth of both species. Differentiation between MRSA and *S. intermedius* is an extra and important problem not encountered in human laboratories

Oxoid recently introduced their chromogenic media (Oxoid Chromogenic MRSA agar), which supports the growth of MRSA organisms. A small trial was conducted to assess if this media also supported the growth of *S. intermedius* and whether the use of this media would aid the rapid differentiation in the laboratory of *S. intermedius* and MRSA isolates

Initial work shows *S. intermedius* grows poorly or not at all on this media, those that do grow do not show chromogenic changes typical of MRSA, and potential MRSA are readily identified in mixed cultures from veterinary samples

This media shows promise for the routine identification of MRSA from veterinary samples and in the screening of large populations in carriage studies

The use of the chromogenic media increases costs but this has to be balanced against the cost of an API Staph or similar biochemical system and the missing of an isolate.

References

Cowan and Steel. Manual for the identification of medical bacteria

Guidelines for the Laboratory Diagnosis and Susceptibility Testing of Methicillin-Resistant *Staphylococcus aureus* BSAC

ESVD Workshop on cutaneous immunology

P11 The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage in healthy dogs and cats, and the recovery from environmental surfaces in private companion animal practices in Southern Ontario, Canada

Colleen Murphy¹, Richard Reid-Smith^{1,2}, Patrick Boerlin³, John Prescott³, Brenda Bonnett¹, Nicol Janecko¹ Scott McEwen¹ and Scott Weese⁴

¹ Department of Population Medicine, Ontario Veterinary College, University of Guelph

² Laboratory for Foodborne Zoonoses, Public Health Agency of Canada

³ Department of Pathobiology, Ontario Veterinary College, University of Guelph

⁴ **Clinical Studies, Ontario Veterinary College, University of Guelph,**

Most infections of methicillin-resistant *Staphylococcus aureus* (MRSA) in dogs have been in dogs with recent veterinary hospital contact. Zoonotic transmission of MRSA between people and pets has been demonstrated in the community and within a veterinary hospital environment¹. The identified strains of MRSA associated with canine disease are the prevalent strains of MRSA associated with human disease¹. To further understand the epidemiology of MRSA, studies were conducted whose objectives were to 1) identify the prevalence of carriage of MRSA in healthy dogs and cats; and 2) describe the environmental recovery of MRSA (EN-MRSA) from surfaces and equipment within veterinary practices in Southern Ontario.

Methods

In 2002 a study was conducted examining the carriage of resistant bacteria by healthy dogs and healthy cats at private veterinary practices. Samples were collected from the perineal region using a standard, sterile bacterial culturette. In 2004 a study examining the environmental recovery of zoonotic and nosocomial pathogens from private veterinary practices was conducted. Environmental samples were collected using sterile Swiffer[®] cloths.

Results

The prevalence of MRSA carriage in healthy dogs and cats was 0%. The prevalence of recovery of MRSA in private veterinary clinics was 10% (tables - 4%; floors - 3%; telephones, keyboards and taps - 2%; kennels, isolation, stethoscopes, otoscopes, ophthalmoscopes - 1%). There were no associations between EN-MRSA recovery and specific disinfectant products, hand-sanitizers or injectable antimicrobials. There was a protective association between the use oral amoxicillin-clavulanic acid (AMC) for in-hospital patients and EN-MRSA. In-hospital use of oral doxycycline was identified as a risk factor for EN-MRSA.

Discussion

The prevalence of MRSA carriage in healthy dogs and cats in Southern Ontario is low. However, the prevalence of recovery of MRSA from veterinary clinics was higher than expected, suggesting a breach in infection control practices. Although there were no associations between EN-MRSA and disinfection products and hand-sanitizers, other infection control practices, including selection of disinfection products and hand hygiene techniques, barrier protective measures and patient placement, were not evaluated. The protective association between the use of oral AMC and EN-MRSA is difficult to explain. This association may be spurious or the use of AMC correlated with risk factors that were not evaluated. Since the environmental burden is higher in private veterinary practices than might be expected, education of veterinarians about the MRSA risk to their patients and staff, and the infection control practices required to reduce the risk of colonization or infection is required.

References

- 1) Weese JS, Dick H, et al. 2005. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. Vet Micro In press.

P12 Evaluation of Coagulase Positive Staphylococcal Carriage in People and their Household Pets

B Hanselman, SA Kruth, JS Weese.

Dept of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

There is increasing evidence of transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) between people and household pets. The dynamics of transmission of organisms such as staphylococci between people and their pets are poorly understood. It is possible that interspecies transmission of staphylococci is a common but unrecognized occurrence in households, and transmission of MRSA is just a reflection of that. This study evaluated the prevalence of carriage in people and their household pets (dogs and cats) with coagulase positive staphylococci, and the incidence of concurrent colonization of people and their pets with the same staphylococcal species.

Nasal swabs were collected from people, and nasal and rectal swabs were collected from pets in households in Ontario, Canada. A questionnaire was also administered. Enrichment culture was performed and coagulase positive staphylococci were identified via standard methods. Speciation was performed using *S. aureus* LAT, biochemical tests and polymyxin B susceptibility. Methicillin-resistance was evaluated via PBP2a LAT and E-test.

120 households, containing 234 humans, 130 dogs and 157 cats were enrolled. *S. aureus* was isolated from 67 (29%) humans, 19 (15%) dogs and 7 (4.5%) cats. Eight human (12%), and two (11%) canine isolates were MRSA. *S. intermedius* was isolated from 10 (4%) humans, 61 (47%) dogs and 11 (7.0%) cats. Isolates from one human (10%), six (9.8%) dogs and two (1.3%) cats were methicillin-resistant. *S. schleiferi* was isolated from one (0.8%) dog but no humans or cats. This isolate was susceptible to methicillin. Ten (7.7%) dogs and one (0.6%) cat harboured more than one species. Concurrent human-animal colonization with *S. aureus* was present in 10 (8.3%) households, which was 20% of households with colonized humans. *S. intermedius* was found in both people and their pets in six (5%) households, 67% of those with colonized humans.

The prevalence of *S. aureus*, *S. intermedius* and MRSA carriage in dogs was unexpectedly high. The incidence of carriage in dogs with more than one staphylococcal species was also higher than anticipated.

While these results do not confirm intra-household transmission of staphylococci, the relatively high incidence of concurrent carriage in people and their pets of *S. aureus*, and particularly *S. intermedius*, indicates that further study of interspecies transmission of staphylococci in household is required. This is of particular concern with MRSA, and further supports other studies suggesting that MRSA may be transmitted frequently within households. Typing of isolates will help determine whether there was potentially interspecies transmission in these households.

P13 Risk factors for community-associated methicillin resistant *Staphylococcus aureus* carriage in horses presented to a veterinary teaching hospital

JS Weese, Dept of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

Recent studies have indicated that methicillin-resistant *Staphylococcus aureus* (MRSA) carriage is endemic in some segments of the horse population. Infected horses are of concern because of the potential for subsequent development of clinical infection and for the potential to transmit MRSA to other horses or humans. This concern is heightened in a veterinary teaching hospital where large numbers of personnel contact large numbers of horses that may be more susceptible to development of infection. Outbreaks of MRSA infection and carriage have been identified in veterinary teaching hospitals and zoonotic infections have developed.

Part of the MRSA control program at the Ontario Veterinary College involves screening of horses by collection of nasal swabs upon admission, weekly during hospitalization and upon discharge. Because this program currently screens all horses, it is expensive and perhaps inefficient as it may screen large numbers of low-risk horses. However, risk factors for community-associated (CA) MRSA colonization have not been reported for horses. The objective of this study was to identify factors associated with MRSA carriage in horses upon admission to hospital to provide insight into MRSA control program design and the epidemiology of this emerging disease.

MRSA screening program results from Oct 14, 2002 until April 20, 2005 were evaluated. Horses that were colonized at admission were classified as having CA-MRSA colonization. Four controls were randomly selected from horses admitted during the same period and from which a negative admission swabs was obtained. The medical record was reviewed for signalment, historical and clinical information. Age, breed, gender, season of admission, service of admission, admission to the neonatal intensive care unit, farm type, medical procedures performed prior to admission, antimicrobial administration within 30 days of admission, previous hospitalization, previous history of MRSA infection or colonization and previous identification of

MRSA on the farm were evaluated. Stepwise forward logistic regression was used to identify variables that were independently associated with MRSA colonization.

CA-MRSA carriage was identified in 69/3372 (2.0%) horses admitted during the study period. Age less than one month (OR 3.1), Thoroughbred breed (OR 2.6), previous identification of MRSA on the farm (OR 4.7), previous diagnosis of MRSA carriage in the individual horse (OR 33.4) and antimicrobial administration within 30 days of admission (2.6) were all associated with carriage.

An understanding of risk factors for CA-MRSA carriage is important for the design of appropriate infection control programs and to investigate the epidemiology of this important emerging pathogen.

P14 Determination of the prevalence of methicillin-resistant Staphylococci from field pathogenic strains collected from cats and dogs

VALLE Michel, WOEHRLE Frédérique, BOISRAME Bernard, Research centre of Vetoquinol.SA. BP 189, 70204 LURE cedex FRANCE

MRSA are important in medicine due to the risk to human health. Indeed, these strains are very difficult to control and cause many nosocomial infections. MRSA are being reported in animal disease although the source of the infection (animal or human) is not always certain. It is very important to obtain an epidemiological field prevalence of methicillin resistance phenotypes of the staphylococci population isolated from veterinary clinical cases. The strains studied came from the Vetoquinol collection which has established a survey network of pathogenic aerobic strains isolated from canine and feline pathologies.

Screening of pathogenic *S. aureus* and *S. intermedius* strains isolated before treatment in Europe (24 in France, 24 in the Netherlands, 14 in UK and 14 in Germany), between 2002 and 2004 has been carried out according the CLSI (Clinical Laboratory Standard Institute) M31-A2 guideline: (1µg) oxacillin and (5µg) methicillin antibiograms on Mueller-Hinton medium have been used.

The results of zone inhibition diameter of each disk gave the same interpretation of susceptibility.

2 of the 27 *S. aureus* strains tested were methicillin resistant and represent 7.4 % of tested *S. aureus* strains. One strain was isolated from a feline urinary infection in Germany and one was isolated in the Netherlands from a canine otitis infection.

All the *S. intermedius* (49) pathogenic strains tested were methicillin susceptible.

The two MRSA strains did not have the same profiles of resistance:

- one was resistant to penicillin G, ampicillin, amoxicillin, amoxicillin/clavulanic acid, cefalexin, flumequin, enrofloxacin, marbofloxacin, and clindamycin and was susceptible to gentamicin, neomycin, trimethoprim, trimethoprim-sulfamethoxazole, doxycycline and fusidic acid
- the second was resistant to ampicillin and with intermediate resistance to penicillin G, amoxicillin, cefalexin and was susceptible to amoxicillin/clavulanic acid, clindamycin, flumequin, enrofloxacin, marbofloxacin, gentamicin, neomycin, trimethoprim, trimethoprim-sulfamethoxazole, doxycycline and fusidic acid.

All these major antibiotics (amoxicillin/clavulanic acid, cefalexin, doxycycline, fluoroquinolones, gentamicin, sulfamides and fusidic acid) were active against the MSSA strains and against all the *S. intermedius* strains.

S. aureus strains with a methicillin-R phenotype are present in domestic pets. This work should be completed by molecular research of the methicillin gene in order for it to be validated. We do not know the sources of these strains (human or animal) but it seems that a classical antibiogram could be used in veterinary microbiological laboratories to detect the methicillin resistant phenotype strains of *Staphylococcus aureus*. Nevertheless, the major antibiotic families used to treat domestic pets remain active against pathogenic staphylococci strains.

P15 A Pilot Survey of the Prevalence of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) and Methicillin-Resistant Coagulase-Negative Staphylococci (MR-CNS) Nasal Carriage in Small Animal Veterinary Personnel

Deacon V¹, Williams NJ², Dawson S², Pinchbeck G², Roscoe, T² and Hart CA¹

¹ Department of Medical Microbiology

² Department of Veterinary Pathology, University of Liverpool

A small pilot study was undertaken to investigate the prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MR-CNS) in veterinary personnel attending a small animal veterinary conference. Previous surveys have been undertaken to determine nasal carriage of MRSA in veterinary personnel, however these have always been done within the veterinary environment and as such may not reflect actual colonisation of nares, but contamination from the hospital environment.

A study at one small animal UK referral hospital found a very high rate of MRSA mucosal carriage among its veterinary staff (18%) [1], when compared with the reported prevalence in UK community surveys of around 1% [2]. It therefore seems possible that personnel working with animals, for example in veterinary hospitals, could have an increased risk of carrying MRSA. The direction of transmission of MRSA remains unclear. Companion animals may act as reservoirs and transmit MRSA to humans posing a public health risk to owners or veterinary personnel [3, 4]. Alternatively and of concern is that in some cases it may lead to humans carrying MRSA to transmit this to animals. Veterinary staff may be a source of infection within veterinary hospitals and transmission of infection to animals under their care, is of particular concern to veterinary surgeons.

Nasal swabs were taken anonymously from volunteers attending the British Small Animal Veterinary Association (BSAVA) congress. Individuals also completed a small questionnaire to provide information on individuals contact with animals and whether they have been involved in clinical cases with MRSA. Swabs were enriched in nutrient broth containing 6.5% NaCl, before being plated onto mannitol salt agar containing aztreonam and oxacillin-resistance screening agar. All suspect isolates were subjected to Gram-stain, catalase test, staphylase (ProLab Diagnostics) testing, coagulase and antibiotic susceptibility according to BSAC guidelines. All staphylococcal isolates were subjected to PCR assays for both the *mecA* and *femA* genes as confirmation of MRSA and MSSA. Coagulase-negative staphylococci (CNS) were identified using an API20 Staph kit (bioMerieux).

Fifty-one individuals provided nasal swabs, of which 5 (10%, 95% CI:3-21%) were positive for MRSA, all of which were from veterinary surgeons. MRSA isolates were all positive for *mecA*, *femA* and were SCC*mec* IV and were either identical or closely related to EMRSA-15 except for one isolate which had >4 bands different on macro-restriction PFGE. All isolates were negative by PCR for the *pvl* gene. Four (8%) of the swabs were also positive for MR-CNS, of which two were found to carry SCC*mec* I (one isolate identified as *S. simulans*), and two were found to carry SCC*mec* IV (one isolate identified as *S. epidermidis*). Ten (20%) swabs were also positive for methicillin-susceptible *S. aureus* (MSSA).

This small pilot study has demonstrated a higher prevalence (10%) of MRSA nasal carriage in veterinary staff compared to less than 1% estimated for the general population [2]. All isolates except for one were identical or closely related to EMRSA-15, the predominant epidemic strain in the UK, which is also commonly isolated from small animals in the UK. Furthermore some nasal swabs were also positive for both MSSA and MR-CNS with SCC*mec* cassettes, which are commonly found in MRSA and could therefore represent a reservoir of SCC*mec* that could be transferred to MSSA.

References

Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, Dalgaard A, Smith H, Stevens K and Lloyd DH (2005).

Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. J.Antimicrob.Chem.56:692-697.

Abudu L, Blair I, Fraise A and Cheng KK (2001). Methicillin-resistant *Staphylococcus aureus* (MRSA): a community based prevalence survey. Epidem. Infect. 126(3): p. 351-356.

Weese, J.S., et al., *Methicillin-resistant Staphylococcus aureus in horses and horse personnel, 2000-2002*. Emerging Infectious Diseases, 2005. 11(3): p. 430-435.

Hartmann FA, Trostle SS, and Klohn AAO (1997). Isolation of *methicillin-resistant Staphylococcus aureus* from a postoperative wound infection in a horse.

J.Am.Vet.Med. Assoc. 211(5):1158-61.

P16 Dairy Cows – A Possible Source of Methicillin-Resistant *Staphylococcus ssp.*?

Kemp, R., O'Connor, R., Cabell, E.J., Williams, N.J.

Faculty of Veterinary Science, Leahurst, Chester High Rd., Neston, S.Wirral CH64 7TE. e-mail rkemp@liv.ac.uk

Staphylococcus spp. are commonly found on the skin and within the mammary glands of dairy cattle and may result in mastitis. Methicillin-resistant *S. aureus* (MRSA) have been isolated from dairy cattle, but limited data are available on their prevalence. However, a variety of β -lactam antibiotics are routinely used in dairy cattle, representing a potential selection for methicillin resistance. The potential risk for transfer of resistant *Staphylococcus* spp. between humans and cattle, particularly lactating cows, is clear. As well as the farm staff having close contact with the cattle, consumption and handling of unpasteurised milk products is common.

A study was carried out investigating the prevalence of commensal MRSA and methicillin-resistant coagulase-negative *Staphylococcus* spp. (MR-CNS) in dairy cattle. Cows were sampled on 15 dairy farms in Northwest England. Milk samples and udder skin swabs were taken from lactating cows in the parlour during milking, and from the skin of the nares at the time of routine veterinary visits. Milk was aseptically sampled from one randomly selected quarter per cow. Data were recorded on mastitis cases and on farm management practices.

Swabs were plated onto two types of media, oxacillin-resistance screening agar (ORSA) and mannitol salt agar (MSA). Presence of the *mecA* gene was confirmed by PCR and isolates were examined for antibiotic susceptibility according to BSAC guidelines. A total of 1043 samples were examined from 818 cows: 189 milk samples, 101 nasal swabs and 753 udder swabs. MRSA was not found in any samples. However, 17/818 cows were found to be positive for MR-CNS, 12 from udder swabs and 5 from nares swabs.

All milk samples were negative. No association was seen between any of the management factors examined and the prevalence of MR-CNS on a farm. Antibiotic sensitivity testing showed widespread resistance in the MR-CNS isolates to antibiotics commonly in use on dairy farms.

P17 A Survey of Methicillin Resistant *Staphylococcus Aureus* (MRSA) and Methicillin Resistant Coagulase Negative Staphylococci (MR-CNS) in UK Equine Hospitals

Meehan LJ¹, Williams NJ¹, Parkin T², Rendle D³ and Ricketts S⁴.

¹ Faculty of Veterinary Science, University of Liverpool, Leahurst

² Animal Health Trust, Newmarket, UK

³ Liphook Equine Hospital, Liphook, Hampshire

⁴ Rossdales and Partners, Newmarket, UK

A survey was undertaken at three large UK equine hospitals to determine the prevalence of MRSA and MR-CNS. Nasal swabs were collected during an eight-week period from 23 May 2005 to 15 July 2005. Horses were swabbed within 24 hours of admission and during the 24 hours prior to discharge.

Samples were cultured on MSA and ORSA; colonies were selected on the basis of colonial morphology for resistance screening. Cell lysates were prepared for all methicillin-resistant isolates, PCR assays were performed for *femA* and *mecA* genes to confirm speciation and SCC*mec* cassette typing was carried out.

Six-hundred and thirty-three samples were collected from 365 horses. MRSA was not isolated from the samples. MR-CNS was isolated from 221 of the 365 horses sampled (60.5%). Forty-eight percent of horses (177/365) were positive on arrival and 12% of horses (44/365) were positive on discharge, having been negative on arrival. SCC*mec* cassette typing of 179 isolates from 2 hospitals revealed cassette types distinct from those found in MRSA, in all but three cases. The number of days of hospitalisation was associated with the likelihood of being MR-CNS positive on discharge.

Failure to isolate MRSA indicates that the prevalence of MRSA in the UK equine population may be lower than previously estimated. Nevertheless, an average of 2 clinical cases has been reported each year from the hospitals included in the study. The isolates from these cases had distinct MLST types from current human epidemic MRSA. This may indicate a reservoir of different MRSA strains within the UK equine population. The high prevalence of multi-drug resistant MR-CNS is of concern. It is possible that MR-CNS could play a greater role in nosocomial infections in equine hospitals, and act as a reservoir for resistance genes that could be passed to sensitive bacteria.

List of Participants

- **Abbott, Ms Y**
School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Dublin, Ireland.
Yvonne.Abbott@UCD.ie
- **Anderson, Dr M**
Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Ontario, Canada.
mander01@uoguelph.ca
- **Andersson, Dr U G**
National Veterinary Institute, Uppsala, Sweden.
ulrika.grenlund-andersson@sva.se
- **Armstrong, Ms S**
Institute of Comparative Medicine, University of Glasgow.
- **Bagcigil, Dr A F**
Veterinary Faculty, University of Istanbul, Turkey.
fbagcigil@yahoo.com
- **Baptiste, Dr K E**
The Royal Veterinary and Agricultural College, Denmark.
keb@kvl.dk
- **Beck, Miss S**
Nationwide Laboratories, Poulton-Le-Fylde, Lancashire, UK.
sbeck@nwlab.co.uk
- **Bennett, Prof M**
Department of Veterinary Pathology, University of Liverpool, Neston, UK.
molvet@liv.ac.uk
- **Bostock, Dr J**
Royal Veterinary College, Hawkshead Lane, Hatfield, Herts.
elysium1@aol.com
- **Broadwith, Miss H**
Diagnostic Bacteriology, VSD, Dundonald, Northern Ireland.
helen.broadwith@dardni.gov.uk
- **Cheetham, Mr S**
RSPCA, Southwater, West Sussex, UK.
scheetham@rspca.org.uk
- **Clegg, Prof P D**
Equine Studies, University of Liverpool.
P.D.Clegg@liv.ac.uk
- **Corkill, Dr J**
Dept of Medical Microbiology, University of Liverpool.
- **Cuny, Ms C**
Robert Koch Institute, Wermigerode, Germany.
cunyc@rki.de
- **Dallas, Mr B**
Vetoquinol Uk Ltd, Buckingham, UK.
- **Dawson, Dr S**
Faculty of Veterinary Science, University of Liverpool, Leahurst, Wirral.
s.dawson@liv.ac.uk
- **Deacon, Ms V**
Dept of Medical Microbiology, University of Liverpool.
- **Dosher, Mr M**
The Bella Moss Foundation, Edgware, Middlesex.
mark.dosher@ntlworld.com
- **Enright, Dr M C**
Dept of Infectious Disease Epidemiology, Imperial College, London.
m.c.enright@imperial.ac.uk
- **Field, Mr C**
Bayer Animal Health, Newbury, Berkshire, UK.
animal.health@bayer.co.uk
- **Gayford, Mr P**
DEFRA, Page Street, London, UK.
- **Goulding, Ms C**
BVNA, 36 Bolton Road, Chorley, Lancashire, UK.
ctgoulding@tiscali.co.uk
- **Goodyear, Dr K**
Veterinary Medicine Directorate, Addlestone, Surrey
s.ward@vmd.defra.gsi.gov.uk
- **Graham, Dr R**
Dept. of Medical Microbiology, Royal Liverpool Hospital, Liverpool.
bob.graham@rlbuhy.nhs.uk
- **Guardabassi, Dr L**
Dept. of Vet Pathology, Royal Vet and Agricultural College, Frederiksberg, Denmark.
lg@kvi.dk
- **Hanselman, Dr B**
Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Ontario, Canada.
bhansel@uoguelph.ca

- **Hart, Prof C A**
Dept of Medical Microbiology, University of Liverpool.
C.A.Hart@liv.ac.uk
- **Hawkey, Prof P M**
Health Protection Agency, Bordesley Green East, Birmingham.
jane.moore@heartofengland.nhs.uk
- **Heller, Dr J**
Institute of Comparative Medicine, University of Glasgow.
j.heller@vet.gla.ac.uk
- **Hillier, Dr A**
Vet Teaching Hospital, Ohio State University, USA.
hillier.4@osu.edu
- **Hooker, Mr R**
PDSA, Priorslee, Telford, UK.
hooker.richard@pdsa.org.uk
- **Houston, Mr C**
DEFRA, Page Street, London, UK.
- **Hume, Ms L**
VPU, Easterbush Veterinary Centre, Roslin, Scotland.
Laura.Hume@ed.ac.uk
- **Jacques, Miss W**
Alder Veterinary Hospital, Liverpool, UK.
- **Jolliffe, Miss T A**
University of Surrey/ Surrey and Sussex NHS Trust, Fetcham, Surrey, UK.
batnurse2004@tiscali.co.uk
- **Johns, Mr S**
Pro-Lab Diagnostics UK, Neston, Wirral
- **Jorgensen, Dr H J**
National Veterinary Institute, Oslo, Norway
hannah.jorgensen@vetinst.no
- **Jury, Ms F**
Stopford Building, University of Manchester, Manchester, UK.
francine.jury@manchester.ac.uk
- **Kearns, Dr A M**
LHCAI Centre for Infections, Health Protection Agency, London.
angela.kearns@hpa.org.uk
- **Kemp, Dr R**
Dept of Vet Pathology, University of Liverpool.
rkemp@liv.ac.uk
- **Kilpatrick, Ms A**
MAST Group Limited, Bootle, Merseyside
akilpatrick@mastgrp.com
- **Kotarski, Dr S**
Pfizer Animal Health, Kalamazoo, Michigan, USA
susan.f.Kotarski@pfizer.com
- **Laurence, Mr C J**
Dogs Trust, 17 Wakley Street, London
chris.laurence@dogstrust.org.uk
- **Lefebvre, Dr S**
Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Ontario, Canada.
slefebvr@uoguelph.ca
- **Leonard, Dr N**
School of Agr. Food Science & Vet med., University of Dublin, Ireland.
nola.leonard@ucd.ie
- **Lindsay, Dr J**
Centre of Infection, St George's, University of London.
jlindsay@sgul.ac.uk
- **Lloyd, Prof D.**
Royal Veterinary College, Hawkshead Lane, Hatfield, Herts.
david-lloyd@ntlworld.com
- **Loeffler, Dr A**
Royal Veterinary College, Hawkshead Lane, Hatfield, Herts.
aloeffler@rvc.ac.uk
- **Lukuman, Mr A**
Ijebu, Ogun State, Nigeria
alvconsult_nga@yahoo.com
- **Martinan, Dr F**
Laboklin Grish Co KG, Badkissingen, Germany.
buchhaltung@cabohlin.de
- **Maryniak, Ms J**
VNJ, JCA Group, Needham Market, Suffolk, UK.
jenna@jkagroup.com
- **McLean, Mrs C L**
Croft Veterinary Surgeons, Blyth, Northumberland, UK.
louise.mclean1@btinternet.com
- **Michalopoulou, Dr E**
Dept of Vet Pathology, University of Liverpool

