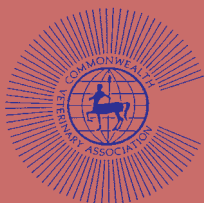


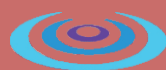
# Commonwealth Veterinary Journal

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The Editor

Commonwealth Veterinary Journal  
 123, 7th 'B' Main Road, 4th Block (West),  
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# Commonwealth Veterinary Journal

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6. Homberger FR. Mäusehepatitis-Virus. *Schweiz Arch Tierheilkd* 1996; 138: 183-188.
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### Acknowledgments

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~ Editor, CVJ

## President's Column



*Dr. S. Abdul Rahman*

*This issue of the **Commonwealth Veterinary Journal (CVJ)** will be the first issue as an e-journal and sincerely hope that our readers will like and appreciate its contents.*

*Midway through 2014, CVA is actively engaged in preparing for the 6th Pan Commonwealth Veterinary Conference (PCVC6) to be held in March 2015 in Kuala Lumpur, Malaysia. A draft scientific programme has been drawn and Prof. Hair, Dean, Universiti Malaysia is attending to fine tuning it before it is finalised and hosted on the CVA website.*

*We the Officers of CVA namely, Dr. Karen Reed, Secretary; Dr. Peter Thornber, Treasurer; Dr. Bob McCracken, Programme Director and self met in London on 27th June 2014 and critically reviewed the plan of work of the CVA and the future programmes especially the CPD programme in Africa. We also discussed the financial situation of the CVA and the urgent need to augment our funds through corporate sponsorship.*

*Making use of the opportunity of being in London we called upon the President of British Veterinary Association Dr. Robin Hargreaves and briefed him about the activities of CVA and extended an invitation to him to participate in the PCVC-6 next year.*

*We also visited World Animal Protection (WAP) formerly WSPA and had detailed discussions with their executive on the role of WAP in CVA projects and programmes. WAP has been generous to offer support to PCVC-6.*

*During my visit to Paris for the Animal Welfare Meeting, Dr. Peter Thornber, Dr. David Bayvel (WAP) and I met Dr. Bernard Vallat, Director General of OIE and requested him to provide OIE support to PCVC-6 and also invited him to the conference which he has graciously accepted. We have since then received confirmation of the support of OIE to the Conference.*

*The Veterinary Association of Malaysia (VAM) has engaged the services of an events management team to organise this mega event. Members of VAM, headed by our Regional Representative of Australasia Oceania region Dr. Paul Chelliah who is also the President of VAM are leaving no stone unturned to host a memorable conference. They will be supported by various organisations both in Malaysia and from abroad in their efforts.*

*Much is yet to be done and the next 8 months will be very crucial for CVA in making sure that the conference will be a great success.*

*I would like to take this opportunity to thank each and everyone who is supporting CVA to make this happen.*

*July 2014*

**S. Abdul Rahman**  
*President*



## Prevalence Of *Listeria* spp. In Raw And Heat Treated Ready To Eat Dairy Products

JKH Ubeyratne, MDN Jayaweera, and KHDT Kasagala

Central Veterinary Investigation Center

Veterinary Research Institute, Gannoruwa, Peradeniya

Sri Lanka

### Abstract

Listeriosis is considered to be a serious infectious disease due to its high mortality rate (30%). Being ubiquitous bacteria, the occurrence of *Listeria* spp in raw milk is unavoidable. *Listeria monocytogenes* which is one of the major foodborne pathogens in human connected with the consumption of dairy products made from unpasteurized milk. In the process of production of milk and dairy products, *Listeria monocytogenes* most commonly occurs as a consequence of post-pasteurization contamination. This bacterium has the ability to multiply and grow at low temperatures (4°C) and to survive even at freezing temperatures. Therefore, in the present study, different heat treated dairy products were analyzed to determine the prevalence of *Listeria* spp. and the occurrence of *Listeria monocytogenes* in ready to eat milk products. Pre enrichment and selective plating followed by molecular typing were carried out for the isolates recovered from 25 raw milk and 68 heat treated ready to eat milk products. Sterilization (in bottle) and UHT treatments have achieved commercial sterility in comparison to pasteurization as shown by 13.23 % (9/68) of *Listeria* spp prevalence in just-after pasteurization and pasteurized commercial liquid milk. No *Listeria monocytogenes* found in all the analysed raw and heat treated dairy products. Zero prevalence of *Listeria* spp. in curd and yoghurts has proven the reduction of viability of *Listeria* spp. at acidic media in curd and yoghurt products.

**Keywords:** Dairy products, Pasteurization, *Listeria monocytogenes*, PCR

### Introduction

Listeriosis remains of great public health concern due to its high mortality rate (20-30%) in immune-

compromised people (Mead et al., 1999). Though the prevalence is low (0.4-0.8/100000) various outbreaks of listeriosis were reported in industrialized countries of Europe and USA associated with dairy, vegetables and meat product consumption (Roberts, 1994). However, dairy products and their processing environments present high potential of exposure to *Listeria monocytogenes* (Luminia et al., 2005).

Genus *Listeria* consists of six species as *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii* and *L. grayi*. Of which only *L. monocytogenes* and *L. ivanovii* are pathogenic (Swaminathan, 2001). While *L. monocytogenes* infects both man and animals, *L. ivanovii* is principally an animal pathogen that rarely occurs in man (Low & Donachie, 1997).

In terms of disease severity *L. monocytogenes* far exceeds other common foodborne pathogens such as *Salmonella enteritidis* (with a mortality of 0.38%), *Campylobacter* species (0.02–0.1%) and *Vibrio* species (0.005–0.01%) in immune-compromised people (Altekruse et al., 1997; Mead et al., 1999). The primary source of infection for both sporadic and epidemic human listeriosis cases is ingestion of contaminated foods (Schlech et al., 1983).

Isolation of listeria colonies from food matrices and identification at species level are time consuming and laborious (Rapporti ISTISAN, 1996). Though the PCR based detection systems are sensitive and specific (Makino et al., 1995; Lantz et al., 1994) direct detection of pathogens in foods has been limited due to its complex composition which contain inhibitors for PCR amplification (Rossen et al., 1992; Bickley et al., 1996).

The aim of this study was to determine the occurrence of *Listeria monocytogenes* in raw and heat treated ready

to eat milk products combining enrichment method followed by isolation on specific plate media and confirmation by PCR.

## Materials and Methods

### *Listeria* spp. Isolation and Identification

25 ml of liquid milk (25g of yoghurt, curd) sample was mixed with 225 ml of *Listeria* enrichment broth (Oxoid CM 862 & Oxoid SR 141) and homogenized using stomacher machine for 3 minutes. Samples were incubated at 37°C for 24 hrs. 0.1 ml of broth was plated on to *Listeria* selective agar (Oxoid CM 856 & Oxoid SR 140, Agar *Listeria* Ottaviani and Agosti) and incubated at 37°C for 48 hrs. Typical colonies were sub cultured on blood agar and incubated at 37°C for 48 hrs. Haemolytic colonies were further tested for gram staining, catalase test, oxidase test, motility test, VP, Urease, Nitrate reduction, Mannitol, Rhamnose, Xylose

### DNA Extraction and Amplification

Presumptive colonies *Listeria* selective media and reference culture (Received from Medical Research Institute, Sri Lanka) which grown on same media were subjected to crude DNA extraction. DNA of isolates was extracted by boiling method. A loopful of bacterial growth was suspended in 0.5 ml of sterile distilled water, boiled for 5 minutes and centrifuged at 10000 rpm for 5 minutes. The supernatant of DNA preparations were stored at -20°C and analyzed for *Listeria monocytogenes* using species specific primers

PCR analysis was performed using primers specific for *Listeria monocytogenes* species, forward 5'CGAATCTAACGGCTGGCACA3' reverse 5'GCCCAAATAGTGTCCACCGCT3', 25µl reaction volume contained H<sub>2</sub>O 7 µl, 2X reaction mix 12.5 µl

(Invitrogen, USA), Taq DNA polymerase 1 µl (Invitrogen, USA), DNA 2.5 µl and each primer 1 µl. Thermal cycling programme consisted with pre cycle at 97°C 5 min, 50°C 1 min, 74°C 2 min followed by 30 cycles at 94°C 30 seconds, 50°C 1 min, 74°C 1 min with final extension 74°C 5 min. 10 µl of PCR products were separated in a 1% agarose gel by electrophoresis and visualized by staining agarose gel with 6 µl ethidium bromide under UV light.

## Results

Initial identification of *Listeria* spp from raw, just-after pasteurization, pasteurized commercial, sterilized (in bottle), UHT milk and yoghurt, curd products were based on the characteristic appearance of colonies on *Listeria* selective agar, gram staining and biochemical test results. Prevalence of *Listeria* spp in pasteurized milk products were 13.23 % (9/68) and that was only in just-after pasteurized and pasteurized commercial liquid milk while 20% (5/25) prevalence was reported in raw milk as shown in Table 1.

### Molecular typing of *Listeria* spp.

All the *Listeria* spp. isolated were typed to determine the prevalence of *Listeria monocytogenes* and the tested samples were negative for *Listeria monocytogenes*

## Discussion

The source of initial contamination of raw milk with *Listeria* spp (20%) could be due to the contamination through environment, gastrointestinal tract and skin of the teats of animals. (Harvey and Gilmour, 1992; Sanaa et al., 1996). Though the prevalence of *Listeria monocytogenes* in the raw milk and dairy products tested in this study was zero, studies in other countries (Europe)

**Table 1: Prevalence of *Listeria* spp in Raw and Heat Treated milk products**

Sample type	Raw	Just after Pasteurisation	Pasteurised commercial Liquid Milk	Yoghurt, Curd	Sterilized (In bottle)	UHT
No. of Samples	25	19	7	24	6	12
No. Positive for <i>Listeria</i> spp	5	6	3	0	0	0
Prevalence	20%	8.82%	4.41%	0%	0%	0%



Figure 1: *Listeria* spp on Chromogenic media (Agar Listeria Ottaviani and Agosti)



Figure 2: *Listeria* spp on Chromogenic media (Oxoid CM 856 & Oxoid SR 140)

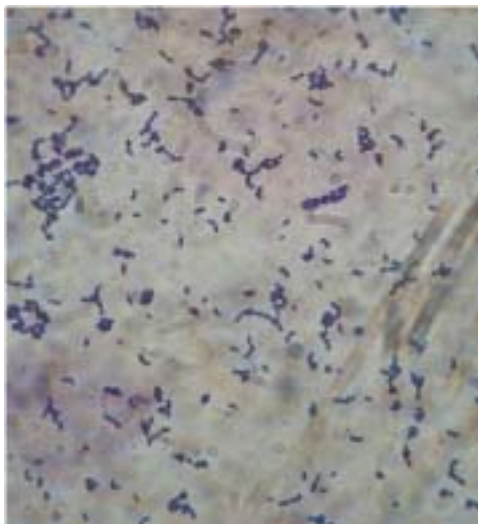


Figure 3: Gram stained smear of *Listeria*

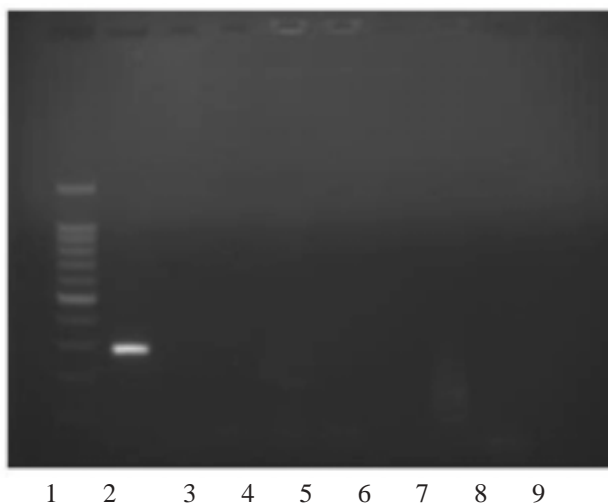


Figure 4: Lane 1: 100 bp DNA Ladder, Lane 2: *Listeria* monocytogenes positive control (287 bp), Lane 3-8: samples, Lane 9: Negative control

have shown that 2,5- 6 % of samples of raw milk can be contaminated with *L.monocytogenes*, indicating potential risk for human population from dairy products manufactured from raw milk (Kozak et al., 1996; Donnelly, 2004). Further, presence of *Listeria* in dairy products such as pasteurized commercial liquid milk (4.41%) and just-after pasteurization of milk (8.82%) can be associated with inadequate pasteurization, post-pasteurization contamination, ability of the organism to multiply during storage at low temperatures, resistance to sanitation preparations (Bottarelli et al., 1999) and recontamination from the equipments used in production (Kasalica et al., 2011).

Time, temperature and initial bacterial load have paramount importance for efficient pasteurization, since temperature of 62.8°C for 30 minutes and 71.7°C for 15 seconds is enough to destroy listeria present in the population of  $10^2$  cfu/ml, but not in the population of  $10^7$ cfu/ml (Jayamanne and Samarajeewa, 2010). However, inability of surviving at temperatures of UHT and sterilization is due to its non-spore forming nature (Todara, 2009) resulting zero prevalence of *Listeria* in UHT and sterilized milk.

Curdling of milk protein in curd and yoghurt is facilitated by adding starter cultures. The acids and

bactericide substances released during starter culture multiplication affect on the viability of *Listeria* (Kozak et al., 1996) and this corroborate the zero prevalence of *Listeria* spp in yoghurt and curd samples tested in the study.

## Conclusion

Destruction of *Listeria* pathogen at different heat treatments are varying and it affects on food safety. Sterilization (in bottle) and UHT treatments have achieved commercial sterility in comparison to pasteurization. Recontamination during pasteurization and cold storage are associated with risk of introduction and multiplication of *Listeria* spp. However, *Listeria monocytogenes* which is the major foodborne *Listeria* spp was not found in this study

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## Adverse Stress Combined *Mannheimia* (*Pasteurella*) *Haemolytica* Outbreak In A Goat Breeding Center In Sri Lanka

*MDA Jayaweera, JKH Ubeyratne, and KHDT Kasagala*  
*Central Veterinary Investigation Center*  
*Veterinary Research Institute, Gannoruwa, Peradeniya*  
*Sri Lanka*

### Abstract

Change from niche of commensal state of *Mannheimia* (*Pasteurella*) *haemolytica* bacteria to pathogenic state, caused by environmental changes lead to death of 4 goats including 2 pregnant ewes and ailing of six adult goats in a breeding center in Central Province of Sri Lanka. The disease condition was diagnosed as pneumonic pasteurellosis caused by *Mannheimia* (*Pasteurella*) *haemolytica* in the investigation based on disease history, postmortem findings, bacteriological tests and the sensitive antimicrobial was selected for the treatments. Clinically diseased animals exhibited severe respiratory signs and distress. Necropsy of two goats demonstrated severe pneumonia, sinusitis and enteritis. The bacterial culture was sensitive to Doxycycline, Tetracycline, Enrofloxacin and resistant to Amoxicillin, Cephalexin, Cloxacillin, Bacitracin, Erythromycin, Flumequin, Gentamycin, Neomycin, Polymixin B, Streptomycin and Sulpha-Trimethoprim.

**Keywords:** *Mannheimia* (*Pasteurella*) *haemolytica*, pneumonia, goats, breeding center, adverse stress

### Introduction

Respiratory infections commonly encountered in goats and sheep are often caused by adverse physical and physiological stress combined with viral or bacterial infections. *Pasteurella haemolytica* or *Mannheimia haemolytica* is known as an opportunistic pathogen and

breakdown of innate pulmonary immune barrier due to stress or initial infections may result release of this organism from its commensal status in the nasopharynx and colonize and proliferate in upper respiratory tract leading to lung tissue damage (Kim *et al.*, 1998). The main stress elements that have been incriminated as predisposing causes are environmental and managerial factors (Thompson *et al.*, 1977).

This organism has been recognized as the principal cause of death from pneumonic pasteurellosis affecting cattle, sheep and goats and septicaemic pasteurellosis in sheep and goats (Janet *et al.*, 2008). Pneumonic pasteurellosis is associated with specific biotypes and serotypes of *Pasteurella haemolytica* in cattle, goat and sheep. Among two biotypes (Type A and Type T) Type A is the most prevalent and is associated with the severe form of pneumonia and causes acute infection in goats of all ages. It occasionally has a systemic form in which the gastro-intestinal tract is the other primarily involved system. Significant decrease of growth performance, low morbidity and high mortality rates have been reported in acute outbreaks in kids. Illness and death also occurred in mature animals (Confer *et al.*, 1990). Goats that survive an acute stage may recover or become chronically infected with reduced lung capacity

Hence the disease is responsible for considerable economic losses to the cattle, sheep and other livestock industries in many parts of the world (Gilmour *et al.*, 1989; Bowl and and Shewan, 2000).

The particular acute and contagious respiratory disease outbreak in goats occurred in one of the three goat breeding centers of the Department of Animal Production and Health in Sri Lanka. The study describes the findings of the investigation.

## Materials and Methods

### General approach to the investigation

A field investigation was carried out at the goat breeding center and history of the disease was assessed. Clinical and postmortem examinations were conducted and samples from both live and dead animals were collected for laboratory investigations. Handlers of the pens were interviewed and information on numbers of cases and deaths, vaccination history, previous treatment were gathered.

### Samples collected

Samples collected from the necropsied goats include lungs, liver, spleen, bone marrow, swabs from sinuses and brain for isolation of the organism.

### Laboratory Diagnosis

**Postmortem Findings:** In the necropsy severe pneumonia, sinusitis, enteritis with paint brush haemorrhages in the intestines was observed.

**Bacteriological Analysis:** Autopsy samples collected from necropsied goats were subjected to conventional bacteriological analysis. Direct culture of samples on blood and MacConkey agar was performed and incubated at 37°C for 24 hrs. Bacterial growths were subcultured to obtain pure colonies, which appeared as Gram-negative short rods after Gram staining. On blood agar, the colony characteristics of pure cultures were examined and recorded. On MacConkey agar, colony growth and lactose fermentation properties were observed and recorded. The biochemical tests for oxidase, catalase, indole, hydrogen sulphide, urease, glucose and sucrose were subsequently performed. Based on growth characteristics and the results of the biochemical tests, isolate was identified.

**Antimicrobial Susceptibility Testing:** Pure colonies of bacteria were grown in tryptone soya broth. Bacterial suspensions were prepared by matching the samples to 0.5 McFarland turbidity standards and were then used to inoculate plates made with Mueller-Hinton media. Antimicrobial discs (Doxycycline, Tetracycline, Enrofloxacin, Amoxicillin, Cephalexin, Cloxacillin,

Bacitracin, Erythromycin, Flumequin, Gentamycin, Neomycin, Polymixin B, Streptomycin and Sulpha-Trimethoprim) were distributed on the inoculated plates using an antimicrobial dispenser and the plates were incubated at 37°C. Results were read after an incubation period of 18 to 24 hours.

## Results and Discussion

### Disease History

The outbreak was first reported in young and pregnant animals especially in the sheds located in hilly terrain just after the rain fall. In sick goats respiratory distress with mucopurulent nasal discharges was the major sign and the disease was associated with sudden environmental changes as Thompson *et al.*, 1977.

### Postmortem Findings

Necropsy examination of two goats revealed severe pneumonia, sinusitis and enteritis.

### Bacteriological Analysis

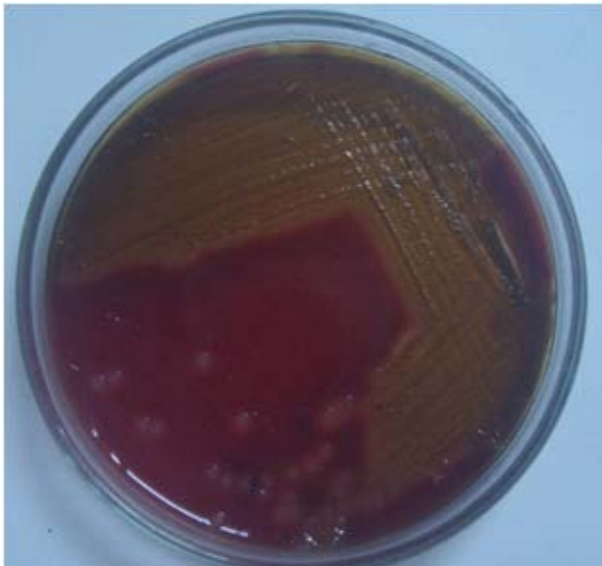
Bacterial isolate which was isolated from brain tissue was identified based on growth characteristics, gram staining and biochemical test results (Fig 1 & 2).

### Antimicrobial Susceptibility Testing

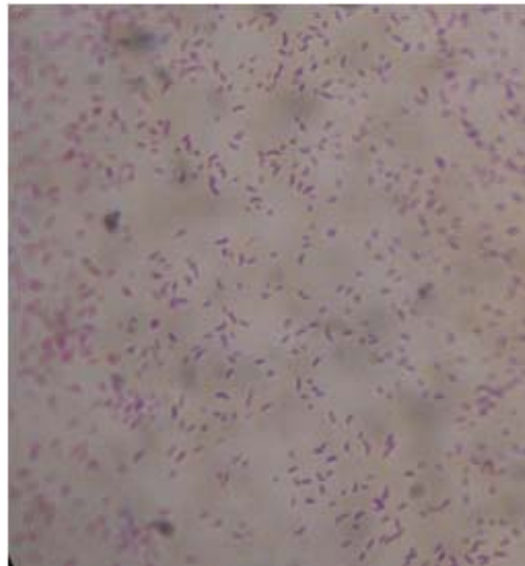
Out of 14 antimicrobials used, the *Mannheimia (Pasteurella) haemolytica* isolate was sensitive only to Doxycycline, Tetracycline and Enrofloxacin and culture was resistant to Amoxicillin, Cephalexin, Cloxacillin, Bacitracin, Erythromycin, Flumequin, Gentamycin, Neomycin, Polymixin B, Streptomycin and Sulpha-Trimethoprim.

## Conclusion

The present information revealed environmental changes are a predisposing factor to release this *Mannheimia (Pasteurella) haemolytica* from its commensal status. Improvement of environmental conditions and management systems will lead to reduction of respiratory disease caused by *Mannheimia (Pasteurella) haemolytica*. Though vaccination against *Mannheimia (Pasteurella) haemolytica* was not generally practiced in Sri Lanka, it will be advantageous to practice vaccination in larger herds as a preventive measure. Development of antibiotic resistance is also an emerging issue and prudent use of antimicrobials is a must.



**Fig 1:** *Mannheimia haemolytica* on blood agar, revealed grey, medium sized colonies with zones of haemolysis



**Fig 2:** Gram stained *Mannheimia haemolytica* revealed gram negative coccobacilli

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## Application of N-Protein Monoclonal Antibody based Direct Fluorescent Antibody Assay (DFA) and Direct Rapid Immunohistochemistry Test (dRIT) for Detection of Rabies Virus in Brain Samples of Animals in India

NithinPrabhu, K,<sup>1,2</sup> Isloor, S,<sup>1,2</sup> Veeresh, BH,<sup>2</sup> Rathnamma, D,<sup>1</sup> Yathiraj, S,<sup>2</sup> Satyanarayana, ML,<sup>2</sup> Placid D'Souza,<sup>3</sup> Neelufar, MS,<sup>1,2</sup> Sharada, R,<sup>1</sup> and Abdul Rahman, S<sup>2,4</sup>

CVA-Crucell-KVAFSU Rabies Diagnostic Laboratory  
Veterinary College, Hebbal, Bangalore  
India

### Abstract

A study was conducted to compare the direct fluorescent antibody (DFA), and direct rapid immunohistochemistry (dRIT) methods of rabies virus (RABV) detection from the suspected rabies samples from various animals. In all, 200 brain samples were collected from various domestic (dog, cat, cattle, buffalo, horse, pig and goat) and wild (wolf and jackal) animals. These samples were collected from different geographical locations / states in India. All the samples were screened for the presence of RABV by employing DFA and dRIT. Totally, 129 of the 200 samples were positive by both the tests, indicating 100 per cent correlation between DFA and dRIT. The dRIT was found to be suitable in the prevailing conditions in India, as it is independent of Fluorescent microscope, incubator and employs tissue fixation using formaldehyde.

### Introduction

Canine rabies is a deadly disease in most developing countries. Worldwide, approximately 55,000 (90% CI: 24,500-90,800) human deaths occur per year. In India, animal and human rabies are endemic throughout the subcontinent with the exception of Andaman and Nicobar Islands. According to the earlier reports, more than 96% of rabies incidences in India are the result of contact with infected dogs. In addition, rabies was also reported through the contact with infected jackals (1.7%), cats (0.8%), monkeys (0.4%), mongooses (0.4%) and foxes (3%) (Bhatia *et al.*, 2004).

Laboratory based confirmatory diagnosis of rabies constitute the most important component of rabies control programs. Most of the diagnostic techniques for rabies

have been standardized internationally. There are neither gross pathognomonic lesions nor specific, constant clinical signs and infallible anti mortem diagnostic tests for rabies, confirmatory diagnosis can only be made in the laboratory. Currently, laboratory techniques are employed on brain tissue from the cranium [especially brain stem, Ammon's horn (Hippocampus), thalamus, cerebral cortex and medulla oblongata]. A composite of such sample should be tested and the brain stem is the most important component of the sample.

The direct fluorescent antibody (DFA) test has been recommended as the gold standard of rabies diagnosis by the WHO (Dean *et al.*, 1996; WHO, 2005). However, higher cost involved in the fluorescence microscopy limits the overall usage of the DFA test in developing countries including India and hence is limited to few regional or state level laboratories. Furthermore, the fixation of the brain sample by chilled acetone does not completely inactivate the rabies virus in the impressions of the DFA slides thereby posing a possibility of biohazard (Umoh and Blendon, 1981). In view of these constraints, the Centre for Disease control and Prevention (CDC), Atlanta, USA have developed a user friendly, N-Protein Monoclonal antibody based direct rapid immunohistochemistry test (dRIT) that could be carried out by fixing the tissue in buffered formalin, incubating at room temperature and using conventional light microscope (available in almost all the local level laboratories) to visualize the inclusions. Due to these major advantages, the dRIT is becoming popular at the global level in detection of rabies viral antigens in the brain specimen. In India, there is scanty information on the application of dRIT on either animal or human samples (Madhusudhan *et al.*, 2012, Chandrasekhara *et*

1 Department of Microbiology

2 CVA-Crucell-KVAFSU Rabies Diagnostic Laboratory

3 Department of Parasitology, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Hebbal, Bangalore, India

4 Commonwealth Veterinary Association



*al.*, 2014). Furthermore, these dRIT based data generated are restricted to specific localities in India. In view of this, the present study was undertaken to compare the performance of dRIT with the gold standard DFA in diagnosing rabies in the suspected brain samples.

## Materials and Methods

### Sample collection

A total of 200 brain tissue samples were examined for rabies virus. Some of the brain samples were collected as whole intact brain and others were collected in parts based on the status of animal at the time of post mortem examination. The brain samples from various animal species including dog (n=161), cow (n=19), buffalo (n=9), horse (n=4), cat (n=3), wolf (n=1), pig (n=1), goat (n=1), and jackal (n=1) were either collected during the postmortem at Dept of Veterinary Pathology, Veterinary College Hebbal, Bangalore, Karnataka (n=91), or were kindly provided by Dept. of Microbiology, College of Veterinary Science, Anand, Gujarat (n=03), the Chief Disease Investigation Office, Palode, Thiruvananthapuram, Kerala (n=51), Bombay Veterinary College, Mumbai, Maharashtra (n=8), Dept. of Veterinary Pathology, GADVASU, Ludhiana, Punjab (n=19), Help in Suffering, Jaipur, Rajasthan (n=3), Dept of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu (n=18) and IVRI, Izatnagar, Uttar Pradesh (n=05) during the period of 2012 up to 2013. Further, a few samples were collected from the field outbreaks from Andhra Pradesh (n=01) as well as from Pondicherry (n=1) (Table 1). The samples were transported to the CVA-Crucell-KVAFSU Rabies Diagnostic Laboratory at Veterinary College, Bangalore. All these samples were subjected to testing by DFA which is considered to be the 'gold standard' and Direct Rapid Immunohistochemistry test (dRIT).

### Direct Fluorescent Antibody Test (DFA)

Initially, impressions of the test brain samples, were made from the brain stem and allowed to air dry for 2 min. The dried impressions were fixed in chilled acetone at -20 °C for an hour and then were removed and air dried. Furthermore, antirabies FITC monoclonal antibody conjugate (Light Diagnostics Rabies DFA III Cat# 6500) along with Evans Blue (0.5% in PBS, Sigma, Product #E-0133) at a final concentration of 0.00125% was added by dispensing through a syringe fitted with a 0.45 µm low protein binding filter. (Note: Tissue when stained with counterstain Evan's blue, notably appears red, and there by provides good contrast with apple green fluorescence

of RABV inclusions, if any. Thus interference in interpretation of the result is avoided due to elimination of green autofluorescence of tissue). The impressions were then incubated for 30 minutes at 37 °C in a high humidity chamber. After staining, excess conjugate was drained from the slides or wicked onto absorbent paper and the slides were briefly rinsed by immersing into the PBS for 3 to 5 minutes. Slides thus washed were carefully blotted to remove excess liquid, briefly air dried before mounting by dropping a small amount of 20% glycerol - Tris buffered saline pH 9.0 onto cover slips and inverting the slides on them. The excess mountant was blotted using filter papers. Further, control impression was prepared using the same tissue and was added with a Rabies Negative Control FITC conjugate (Light Diagnostics TM, # 5102). The control conjugate along with counter stain Evan's blue was diluted in the same manner as done to the anti-rabies conjugate.

### Direct Rapid Immunohistochemistry test (dRIT)

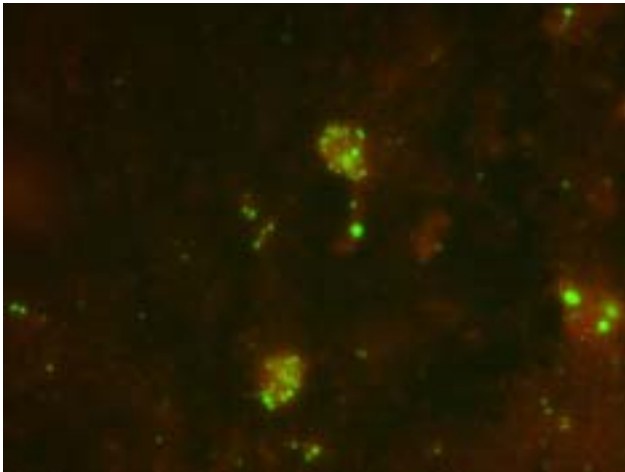
The dRIT was performed using the dRIT kit provided by CDC, Atlanta, GA, USA following the standard instructions. The test was done at the ambient temperature without using the incubator. Touch impressions, were made on glass microscope slides as described earlier. The slides were air-dried, fixed in 10% buffered formalin for 10 min, dip-rinsed in wash buffer PBS with 1% Tween 80 (TPBS), immersed in 3% hydrogen peroxide for 10 min, and dip-rinsed in fresh TPBS. After dipping, the excess buffer was shaken from the slides and blotted from the edges surrounding the impression. This treatment was repeated after each rinsing step. The slides were incubated in a humidity chamber (or a 96 well tissue culture plate cover on a moistened paper towel on an even surface) with the biotinylated monoclonal antibody cocktail for 10 min, dip-rinsed in TPBS, incubated with streptavidin-peroxidase complex (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD, USA) for 10 minutes and dipped in TPBS. A 3-amino-9-ethylcarbazole (AEC) stock solution (available in the kit) was used to prepare working AEC solution. The A working solution was prepared by adding 1 mL AEC stock solution to 14 mL 0.1 mol/L acetate buffer (provided in the kit) and 0.075 mL 3% hydrogen peroxide. The slides were incubated with the AEC peroxidase substrate for 10 min and dip-rinsed in distilled water. They were then counterstained with 1:2 Gill's hematoxylin (available in the kit) for 2 min and dip-rinsed in distilled water. Finally, they were mounted with a water-soluble mounting medium and examined by light microscopy (Carl Zeiss AG, Göttingen, Germany) at magnifications of ×200 to ×400. Further, a test control

**Table 1: State-wise and species-wise details of sample collection**

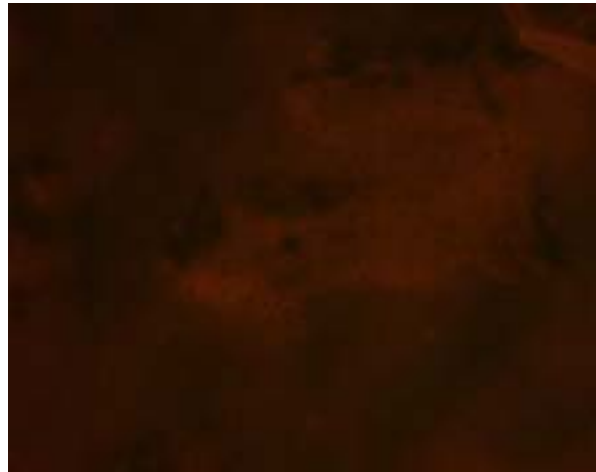
Species	State										Total
	Andhra Pradesh	Gujarat	Karnataka	Kerala	Maharashtra	Pondicherry	Punjab	Rajasthan	Tamil Nadu	Uttar Pradesh	
Dogs	1	-	79	45	8	1	6	3	17	2	162
Cattle	-	2	7	4	-	-	4	-	-	1	18
Buffalo	-	-	1	-	-	-	6	-	-	2	9
Horses	-	-	2	-	-	-	1	-	1	-	4
Cats	-	1	1	1	-	-	-	-	-	-	3
Pig	-	-	-	-	-	-	1	-	-	-	1
Goat	-	-	-	1	-	-	-	-	-	-	1
Jackal	-	-	-	-	-	-	1	-	-	-	1
Wolf	-	-	1	-	-	-	-	-	-	-	1
<b>TOTAL</b>	<b>1</b>	<b>3</b>	<b>91</b>	<b>51</b>	<b>8</b>	<b>1</b>	<b>19</b>	<b>3</b>	<b>18</b>	<b>5</b>	<b>200</b>

**Table 2 : Species-wise Details of the Samples Resourced and their DFA and dRIT**

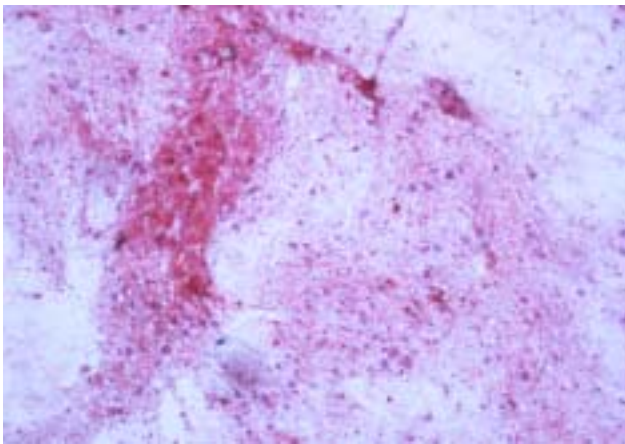
Host	Total No. of samples collected				
	No of Samples collected	Positive by DFA	Positive by dRIT	No of samples unfit for DFA/dRIT	No. of Negative Samples
Dogs	161	101	101	32	28
Cattle	19	14	14	1	5
Buffalo	9	9	9	-	-
Horses	4	2	2	-	2
Cats	3	-	-	2	1
Pig	1	1	1	-	-
Goat	1	-	-	1	-
Jackal	1	1	1	-	-
Wolf	1	1	1	-	-
<b>TOTAL</b>	<b>200</b>	<b>129</b>	<b>129</b>	<b>36</b>	<b>36</b>



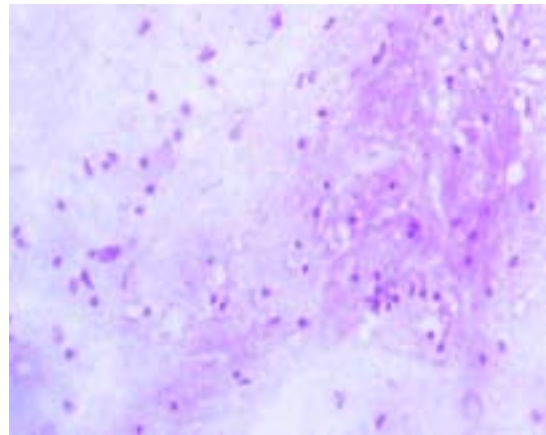
*Fig. 1: A DFA test of a brain stem impression from a rabies virus-infected test sample with anti-N protein Mab - FITC conjugate (Light diagnostics TM, Rabies DFA III, # 6500) showing viral inclusions (200X magnification).*



*Fig. 2: A DFA test of a brain stem impression from a test sample with Rabies Negative Control FITC conjugate (Light diagnostics TM, # 5102) (200X magnification).*



*Fig. 3: A dRIT of a brain stem impression from a rabies virus-infected test sample showing dark red colored deposits of RABV inclusions scattered throughout the impression (200 X magnification).*



*Fig.4: A dRIT of a brain impression from a non-rabid dog showing mere counterstaining (blue) of tissue with Gill's hematoxylin (200 X magnification).*

was maintained by making a healthy tissue impression and processed as above.

## Results and Discussion

The analysis of data from DFA and dRIT revealed that 57 /91 brain samples from Karnataka and 1/1, 1/3, 18/51, 8/8, 1/1, 17/19, 3/3, 18/18, 5/5 samples from Andra Pradesh, Gujarat, Kerala, Maharashtra, Pondicherry, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh respectively were positive by DFA (Fig.1). Further, test control slide with Rabies Negative Control FITC conjugate (Fig. 2) did not reveal any apple-green fluorescence suggestive of RABV antigens.

In toto, 64.50 per cent (129/200) of samples were

positive by DFA (Table 2). However, the samples resourced from the states, Kerala (n=51), Maharashtra (n=8) Punjab (n=19), Tamil nadu (n=18) and Uttar Pradesh (n=05) were previously tested to be positive in the respective state laboratories from where only rabies positive samples were transported to Bangalore.

In the present study, 129 out of the 200 samples were positive by DFA. Of the 129 DFA positive samples, 101 had been previously tested in respective state laboratories and remaining were fresh suspect samples. For this, the brain stems were considered to be the samples of choice. The DFA was carried out according to the protocol described by the CDC, Atlanta, USA. The impressions from all the 129 brain samples showed bright green fluorescent particles of varying sizes either

scattered or within the neurons, mirroring similar descriptions by others (Lembo *et al.*, 2006; Durr *et al.*, 2008; Madhusudana *et al.*, 2012; Chandrashekhara *et al.*, 2014 ; Isloor *et al.*, 2014 and Nithinprabhu *et al.*, 2014). However, 36 of the 200 samples resourced were found to be decomposed to various levels, and consequently produced considerable levels of non-specific tissue fluorescence/auto-fluorescence which made the interpretation ambiguous. This is in line with the earlier findings that there was a considerable loss of sensitivity of DFA, or even false negativity, when decomposed tissues were used (Whitby *et al.*, 1997 and David *et al.*, 2002).

Despite wide approval, the use of DFA has a few limitations. The major drawback is that the acetone as fixative does not completely inactivate the infective virions, posing potential hazard to laboratory personnel (Umoh and Blenden, 1981). Studies have demonstrated that intracerebral inoculation of acetone fixed tissues could cause disease in suckling mice (Umoh and Blenden, 1981). Other constraints are requirement of expensive fluorescent microscope, well-trained personnel and to rule out the background auto-fluorescence that is produced (Malovrh and Hostinik, 2005). Moreover, the test could be subjective, suggesting the need of two independent readers for routine diagnosis (Robardet *et al.*, 2011).

In view of these limitations of DFA, “direct rapid immunohistochemistry test” (dRIT) to detect the RABV antigen was developed at the CDC, Atlanta, USA, (Niezgoda and Rupprecht, 2006). The principle of dRIT is based on the capture of rabies virus nucleoprotein antigen by biotinylated anti-nucleocapsid monoclonal antibodies and colour development by streptavidin-peroxidase and chromogen amino-ethyl carbazole. The test does not require fluorescent microscope and ensures complete inactivation of RABV due to formalin fixation, making it user friendly and biologically safe. In this study, dRIT detected the RABV inclusions in all the 129 samples tested positive by DFA. The positive results could be declared by the presence of dark red to brown colored deposits scattered throughout the impression (Fig.3), which was in agreement with the earlier findings (Madhusudana *et al.*, 2012, Chandrashekhara *et al.*, 2014.). The test control slide did not reveal any dark red to brown coloured deposits suggestive of RABV antigens (Fig. 4). These findings are in agreement with Lembo *et al.* (2006) who evaluated the dRIT under field and laboratory conditions in both glycerol preserved and in frozen brain samples.

In conclusion, DFA, though a WHO approved gold standard test employed for the confirmatory diagnosis of rabies using post mortem brain samples, it's wide usage is restricted due to the reasons cited above. This hampers the routine testing of brain samples at the national level in large scale. These limitations could be overcome by the application of dRIT, which is found to be working on par with the DFA. Perhaps, this is the first laboratory based large scale screening (employing DFA and dRIT) (n= 200) of animal brain samples resourced from different species of animals (dogs, cattle, buffalos) from 9 states and one union territory of India. Our findings unambiguously indicate that the dRIT could be used in all the regional level as well as local laboratories with minimal laboratory infrastructure in India for post mortem confirmatory diagnosis of rabies in animals. Generation of active data based on this practically feasible approach at the national level could add to the data generated at the global level on the usage of dRIT as an alternative to the DFA. This could further strengthen the international efforts in declaration of dRIT as an approved test by the WHO / OIE and also in evolving the strategies for effective control of rabies in India.

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## The Disease Costs of Wildlife Markets - A Perilous Price to Pay

*Jan Schmidt-Burbach\*, Victor Watkins and Neil D'Cruze*  
*The World Society for the Protection of Animals*  
*5th Floor, 222 Gray's Inn Road, London, WC1X 8HB*  
*United Kingdom*

Hundreds of wild animals, stacked in filthy cages all piled on top of each other as far as the eye can see. Stepping into one of the gloomy makeshift internal corridors of the wildlife market, a manic menagerie unfolds before you. Wild fowl frantically flap beside malnourished macaques whilst geckos grapple for space and fruit bat faeces drop through the mesh wire of their cages onto the civets cowering below. The air around you is so thick with dust particles comprised of skin, feathers and scales that you can almost taste the pathogens as you take each breath.

What sounds very much like a grossly exaggerated scene from a Hollywood horror movie is in actual fact a common feature and fact of life in many major urban centres across the world. Every day hundreds of people will visit a wildlife market either intentionally to make a purchase or unintentionally perhaps as part of their commute to work. Irrespective of the reason why people pass through them, wildlife markets are a serious threat from both a veterinary and a human health perspective.

### Cruel Commerce

Wildlife markets, like the one described above, are a regular feature in many developing countries where consumer demand for exotic pets or wild animal products drives the poaching and trade of wild animals. The true number of animals involved is difficult to quantify, but a study conducted on the Indonesian Island of Java during 2009 counted 340 animals across target species (including 133 primates and dozens of parrots) over a period of just three months alone [1]. Disturbingly, the researchers did not include reptiles, amphibians or unprotected species in their study and so the number of animals reported is likely to represent a underestimate of those actually involved.

The authors have visited a number of wildlife markets in different countries across Asia and have been left with severe animal welfare concerns for the animals found there. Cramped cages with no separation between species, food and dead animals left to rot alongside those which are still alive, and constant interaction with traders and customers alike are just some of the stark indicators of compromised animal welfare.

Many of the animals held in these markets often appear to be in poor body condition, but it is important to note that poor animal welfare is not limited to factors which impact only on their physical health but also includes the often overlooked (even by veterinary practitioners) psychological health factors [2].

Stress, for example, is a critical factor contributing negatively to wild animal welfare and needs special consideration for situations where wild animals are kept in captive conditions [3,4]. Undomesticated by very definition, wild animals do not share the same tolerance (or outright lack of fear) of people demonstrated by domesticated species such as dogs and cats. Wild animals are particularly prone to experiencing chronic stress and are thus highly susceptible to infections due to a failure of the immune system to respond appropriately [5,6]. Consequently this leads to a high likelihood of contracting and also spreading disease to both other individuals and other species [10].

### Human Health

In general, the threat of disease spreading between wild animals does not appear to be of major concern to either traders or customers at these wildlife markets. This is of course disturbing from an animal welfare perspective. However, perhaps more surprisingly, the vast majority also do not appear to be concerned about the

\*Corresponding author: [JanSchmidt-Burbach@worldanimalprotection.org](mailto:JanSchmidt-Burbach@worldanimalprotection.org)

ever present threat of contracting one of these diseases themselves. Approximately 60% of emerging infectious diseases (EIDs) are zoonotic and 71.8% of these are thought to originate from wild animals [7]. Consequently, the threat that wildlife markets pose to human health and the important role of veterinarians in minimising this threat is being increasingly recognized [10].

While helminthic infectious diseases are less of a concern in terms of human health, research suggests that viral and protozoan diseases are highly relevant, especially when considering zoonotic EIDs [8]. The reasons for the increasing threat of EIDs to human health have been primarily attributed to factors such as globalization and encroachment on wildlife habitat. However, increasing contact via live animal and bush meat markets, access to petting zoos, and ownership of exotic pets are also thought to be playing a significant role [9].

The location of many wildlife markets in high-risk urban locations means that pathogens have the potential to spread rapidly to market visitors who may then go about their daily lives in highly populated cities bringing them into contact with hundreds more people during the process. The fact that people are able to take flights across more than half the globe in less than 24 hours also highlights the distance and speed at which diseases originating from wildlife markets can achieve.

## Swift Solution

The only way to totally eradicate both the animal welfare and human health threats posed by wildlife markets would be close them. Changing consumer patterns and preventing poaching from the wild are critical in this respect. However, where this may not be immediately possible, there are rapid steps that can be taken to significantly reduce the threat of spreading disease. Rather than attempting to eradicate the pathogens or the wild species that harbour them, it has been suggested that a more practical approach would focus on decreasing chances of contact between species, including humans, especially in the wildlife trade sector [10].

At the same time, regular monitoring of markets for disease occurrence and animal welfare conditions would allow to identify early-warning signs. In fact, due to the close link to likelihood of disease occurrence, the animal welfare conditions could play an especially important role as proxy-indicator for estimating the chance of an occurrence of EID in wildlife trade situations. On a larger regional and global level the success of detection and

control of EIDs will largely be based on international solidarity and cooperation between countries [11].

## A Perilous Price to Pay

In summary, the situation at live wildlife markets across the world is deeply worrying. The catastrophic conditions that wild animals are kept in raise severe animal welfare concerns which are closely linked with concerns around emerging infectious diseases and their wider impact.

In order to totally eradicate the threat, it is advised that national authorities should take measures to end the existence of wildlife markets by addressing consumer demand and effective enforcement. Where closure is not immediately feasible, to address this threat it is strongly advised that responsible authorities should devise guidelines that will decrease the suffering of wild animals by limiting the trade in wild animals, keeping species separated from each other, decreasing interaction between wild animals and people, introducing strict hygienic measures and regular monitoring measures.

To translate these recommendations into practice, it is crucial that members of the veterinary community become aware of the risks posed by wildlife markets and the link between animal welfare and EIDs. These issues should be addressed in veterinary fora or discussions with national and local authorities, and also in everyday interaction with clients. In this way veterinarians can make a fundamental difference for animals' and public health.

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## What makes an excellent vet school? You tell us.

Vet schools do so much for so many – animals and people. And here at **World Animal Protection** we think it's high time that their excellent work was celebrated. That's why we're developing a new set of standards to recognise schools with impressively high levels of animal welfare.

But we need your help.

All we're asking you to do is **fill in this short survey** by 1 September 2014 to help us develop these new standards. This is your chance to have your say about what they should cover, how vet schools should be assessed – and more besides.

As you probably know, guidelines from the World Organisation for Animal Health (OIE) say all new veterinary graduates need a good understanding of animal welfare as a Day 1 Competency. And these new standards will recognise schools whose students achieve exactly this.

All we need now is just 10 minutes of your time. So please, complete our short survey today. And – as a token of our thanks – we'll enter you into a prize draw to win either an animal welfare textbook or a \$10 Amazon voucher.



We were known as **WSFA**  
(World Society for the  
Protection of Animals)





## World Animal Protection

I am delighted to begin this section on Animal Welfare with some very exciting news, WSPA (World Society for the Protection of Animals) has now become World Animal Protection.

While our name has changed, our focus has not - it will remain firmly fixed on our work protecting animals. Around the world, millions of animals endure cruelty and suffering. Protecting animals around the world is our history and our future - and now it is our name too. Changing to World Animal Protection brings our name in line with what we are trying to achieve - protecting the world's animals.

That's why we needed our name to evolve. That's why we need it to be clear, distinct and memorable. And that's exactly what our new name is.

Our new name demonstrates who we are and what we stand for in a clear and simple way. This will help people understand our goals and support us in transforming the lives of billions of animals around the world.

The core purpose of our organisation has not altered, it has strengthened. We always have been - and always will be - about protecting the world's animals. Our history will always be a key aspect of the work we do.

The change is not about forgetting our past. It is about protecting our heritage, by ensuring that a freshly refocused World Animal Protection continues to transform the lives of animals around the world, as we have done for over 30 years.

Every day, in every country, millions of animals are suffering. Our priority is- and always will be - protecting the world's animals.

World Animal Protection will continue to be a global strategic partner in animal welfare for the veterinary profession and build on its established relationship with the Commonwealth Veterinary Association to move the world to protect animals.

In this edition we have three feature articles from the World Animal Protection team. The first article is on Animal Mosaic by Pablo Lalor, External Relations Coordinator, World Animal Protection. Animal Mosaic is an online platform bringing together people working to protect animals around the world. Through this site it is possible to collaborate, download resources and keep up to date with the latest global developments in animal welfare.

World Animal Protection is working with other organisations towards a Universal Declaration on Animal Welfare (UDAW) which we hope to have endorsed by the United Nations. This will be an agreement amongst the world's people and nations acknowledging that animals are sentient, that is, they can suffer and feel pain and therefore animal welfare must be respected. Helen Proctor, Sentience Manager at World Animal Protection in the second article explores in detail what sentience means.

The third article is by Jan Dr. Jan Schmidt-Burbach (Senior Wildlife and Veterinary Advisor, World Animal Protection Asia Pacific), Dr Neil DCruze (Head of Wildlife Research and Policy) and Victor Watkins, Wildlife Adviser. It considers the impact of wildlife markets on the welfare of animals and the disease risk to both humans and animals of this close confinement.

The final information at the end of the articles is about World Animal Protection developing voluntary standards to help veterinary schools demonstrate excellence in applying animal welfare principles. These standards cover ten key areas of teaching, research and organizational culture. World Animal Protection would like to hear from vets, vet educators and veterinary students. The online survey only takes 10-12 minutes: see [insert page no.]for more details.

For further information on any of the areas covered contact the contributors directly, or for general comments and suggestions for future animal welfare related input into this journal then please email, Joe Anzuino on: [JoeAnzuino@worldanimalprotection.com](mailto:JoeAnzuino@worldanimalprotection.com).

## Animal Mosaic: Collaborating Online For Animal Welfare

On Animal Mosaic you can find useful tools to aid with Continual Professional Development (CPD); discover and debate the latest research on animal sentience; and connect with veterinarians and animal welfare professionals from all over the world. The website is an indispensable tool in progressing animal welfare together, for the good of the animals and everyone working in related sectors.

### Your Professional Development

Our understanding of animal health and welfare issues is constantly developing and new tools and technologies are introduced every day. The Education section on Animal Mosaic offers you a 'one stop shop' for CPD in animal welfare. A comprehensive area focussing on tertiary education ([animalmosaic.org/education/tertiary-education/](http://animalmosaic.org/education/tertiary-education/)) contains a wide array of CPD and educational tools for all veterinarians as well as students, including tools to aid welfare assessment. These are sourced by World Animal Protection from all corners of the internet to provide you with the best possible service.

The tools are collated to compliment World Animal Protection's learning resource, Concepts in Animal Welfare. The previous issue of the Journal of the Commonwealth Veterinary Service (January 2014) contained an in depth look at Concepts in Animal Welfare. Whether teaching students or updating your knowledge on animal welfare as a veterinarian, the newly launched 3rd edition is the comprehensive animal welfare syllabus you need. It includes the latest animal welfare research, legal information and teaching examples from all over the world. Academically robust and unbiased, Concepts in Animal Welfare allows you to examine all sides of the ethical debate.

### The Science of Animal Sentience

Our understanding of animal sentience is of paramount importance to veterinary care. As a research topic it is a young discipline, growing rapidly in its body of evidence. To maximise the efficiency of the care of animals, it is essential to integrate the latest knowledge on pain and suffering, as well as emotions such as joy and pleasure. Animal Mosaic contains an in depth analysis of our understanding of animal sentience in the Sentience Mosaic ([sentience mosaic.org](http://sentience mosaic.org)).

World Animal Protection collates the latest research that has a bearing on our understanding of animal sentience and summarises its potential impact. Exploring the 'Knowledge' section of the platform will help you apply this research to your work and professional development. This ever growing section is one to visit regularly to keep informed on cutting edge developments.

Learning from the experts in the field is crucial for developing our understanding of animal sentience. On a monthly basis the Sentience Mosaic opens up animal sentience topics for discussion with these experts. Live online debates hosted on the website connect you, the audience, with other veterinarians, animal welfare professionals and scientists whose work impacts animals to push the conversations around animal sentience forward. Recent debates have included, "What is the vet's role in animal welfare?" and "The science of animal play: can play be used as a welfare indicator?".

### A Global Community

Animal Mosaic is a fully interactive platform visited by thousands of users across the globe. In addition to its resource libraries and learning tools, the website also contains an online community of discussion forums housing over 1,250 registered professional members. They are veterinarians, campaigners and animal welfare professionals, amongst others with a vested professional interest in animal welfare. This ever growing community has representation from over 100 countries worldwide in all regions.

These forums allow members to collaborate online and learn together for the benefit of professional development and ultimately animal health and welfare. Flexible to your needs, users are able to host private discussions or work together openly in a professional environment. To see how the Animal Mosaic Community can benefit your work, join today. It's free and takes less than 2 minutes of your time ([animalmosaic.org/community/](http://animalmosaic.org/community/)).

If you are interested to learn more about Animal Mosaic please visit at [animalmosaic.org](http://animalmosaic.org) or email [pablolalor@worldanimalprotection.org](mailto:pablolalor@worldanimalprotection.org).

~ Pablo Lalor  
External Relations Coordinator  
World Animal Protection

## Animal Sentience

### What is Animal Sentience?

Animal sentience refers to the ability of animals to feel both positive and negative experiences such as pain and pleasure [1]. As veterinarians, you will be fully aware of the complexity of the animal mind and the importance of considering both the physical and mental health of the animals you care for. It is for this reason that animal sentience is of utmost importance and relevance to your work. Understanding how to measure and improve the emotional states of animals is key to ensuring the well-being of the animals you care for. The past 35 years has seen a notable increase in the scientific study of the subjective lives of animals, and the measurement and assessment of animal emotions is increasingly becoming the subject of rigorous scientific study [1,3,4]. As a result, evidence of animal sentience is growing and this has major implications for how we treat animals and for the policies governing their care.

### What Evidence is there for Animal Sentience?

Evidence of animal sentience is firmly based in neuroscience. All vertebrates have a central nervous system and similar major structures and divisions in the brain [5]. In particular, the limbic system, which is responsible for processing emotions, is similar across all vertebrate species [6]. Furthermore, the recently evolved neocortex, which is responsible for cognitive processes, is present in some form in all vertebrate species [7]. Neurons are also similar across vertebrates, and scientists are now finding complex neurons once believed to be unique to humans in several species of cetaceans, primates and elephants [8-10]. For example, cortical spindle cells specialised in emotional processing have been found in humpback whales [8], and macaques have been found to possess mirror neurons that assist in empathic behaviour and learning [9]. In response to this growth in scientific discussion around the subjective experiences of animals a prominent group of cognitive neuroscientists, neuropharmacologists, neurophysiologists, neuroanatomists and computational neuroscientists gathered at the University of Cambridge in July, 2012, to reassess the neurobiological substrates of conscious experience and related behaviours in human and non-human animals. They produced the 'Cambridge Declaration on Consciousness' which declared that the neocortex was not essential for the experience of affective

states. They stated that non-human animals, including all mammals and birds, and other species, including octopuses, possess the neurological substrates required for generating consciousness (Cambridge Declaration on Consciousness, 2012).

Evidence of animal sentience can also be found in the behaviour of animals. Research has repeatedly shown that animals respond to stimuli in a manner that indicates conscious experience [1,11]. The behaviour of animals therefore provides valuable evidence of sentience, particularly for those species where the neurological evidence is lacking. For example, it has been argued that fish are incapable of feeling pain and suffering because they do not possess the regions of the neocortex and mesocortex thought to be responsible for the conscious experience of pain in mammals [12,13]. The behaviour of fish however, suggests that they do feel pain rather than just nociception [14-16]. When a painful solution of bee venom or vinegar was applied to the mouths of rainbow trout, the trout were less likely to be fearful of a novel object that was added to the tank, compared with the control subjects. They also rubbed their lips into the gravel and against the sides of the tanks, and rocked from side to side. These behaviours and the noticeable drop in their attention levels indicated that they were experiencing pain. Furthermore, when given analgesic morphine the behaviours ceased and the trout became fearful of novel objects again [14].

Behavioural studies have also provided insight into the subjective experiences of invertebrates [17]. Invertebrates lack the particular physical characteristics often thought to be responsible or essential for sentience [6,18,19]. As a result they are generally assumed to be incapable of experiencing pain and are treated very differently from their vertebrate counterparts [20]. Legislation protecting invertebrates is very limited around the world, which means that invertebrates can often be treated in ways which would be illegal and inhumane for vertebrates [18,21]. Research into the subjective experiences of invertebrates is increasing however, and the behaviour of a number of species has indicated that they are capable of conscious experience. For example, research has shown that the decapod crustaceans, crabs and crayfish, respond to painful stimuli by learning to avoid it [22,23], and that glass prawns perform pain behaviours such as rubbing [24], and autotomy [19], and respond to analgesics in the same way as vertebrates [24].

## Looking beyond pain

It is widely accepted that animals feel pain, and veterinarians play a key role in minimising the pain experienced by animals in various situations. Decades of research into animal sentience has also shown us that the emotional lives of animals can also be very complex, beyond the primary experience of pain. Animals are capable of experiencing a wide range of emotions and feelings, from fear and grief to joy and excitement. Animal welfare scientists are increasingly recognising that good animal welfare is about more than just freedom from negative states such as pain and fear, and that animals should lead a good life, one which is rich with positive experiences and emotions [25-27]. Therefore, it is the role of the veterinarian, along with animal owners and carers to ensure that negative emotions and experiences are minimised for animals, whilst positive emotions are actively promoted. This is particularly the case for animals in industry, whether research or agriculture. When a veterinarian considers both the physical and mental health of an animal and takes steps towards minimising suffering and promoting positive emotions, then they are truly improving that animal's welfare.

Research into animal sentience is an exciting and growing field, and we are constantly discovering commonalities between humans and animals. For example, research has shown that rats demonstrate empathy towards restrained cage-mates. In an experiment, free rats were found to open the cage for restrained rats, even when social contact was prevented. When chocolate was offered, the free rat would still release the restrained rat and then share the chocolate with them [28]. Mice have also shown empathic behaviour by modulating their pain sensitivity in response to the observation of their cage-mates experience of pain. Mice showed increased pain behaviours when their cage-mate was also given the same painful stimulus, and this was dependent on visual observation [29]. Evidence for empathy in animals has implications for their treatment. For example, in a laboratory setting rat and mouse cage-mates may be unduly distressed by observing the discomfort and suffering of their fellow cage-mate. This also has wide implications for slaughter and painful husbandry procedures, as the observation of others being slaughtered or in pain, may cause unnecessary fear and distress for any observing animals [30].

## Communicating with non-human animals

When it comes to measuring the emotional state of animals, the subjectivity of their experiences poses some problems. We will never know for sure what is going on in another being's mind whether that being is another human or animal, as emotions are personal, subjective experiences. However, despite the fact that humans do not share a universal language with animals, we can still learn to communicate with them, and learn to understand how they communicate with one another [31]. Any animal, whether they are a herd animal or solitary, needs to be able to communicate. Animals do this in a vast manner of ways, through vocalisations, pheromones, body language and facial expressions. There is now an increasing amount of research which is seeking to understand animal communication, and several studies have sought to determine whether any forms of communication reliably communicate emotional state. For example, several studies have looked at whether ear and tail postures are indicative of positive and negative emotional state in sheep and pigs [32-34]. In recent years researchers have also found that rabbits, horses and rodents grimace when in pain, and that these facial expressions can be used to reliably measure the degree of pain they are in [35-38]. Other studies have looked at whether peripheral temperatures indicate emotional state and have provided some promising results [39,40]. The results from these studies offer tangible, practical solutions to access the emotional minds of animals, and with contextual and species-specific knowledge, they can be used to assess the emotional state of animals. This is particularly advantageous to veterinarians, as it offers new tools to assess the welfare of the animals in their care.

Understanding how animals communicate is a key area of focus within animal welfare and veterinary science as it can offer important insight both into their state of welfare, and how to improve it. Preference testing has been successfully used with a number of species to garner insight into their inclinations [4]. The results can often differ from what is expected, as animals will often prioritise social contact over food, or choose different bedding materials than expected [41]. Preference testing therefore, offers a valuable means of communicating with animals. Motivation testing also offers helpful insight into how motivated an animal is to gain access to a particular resource [42]. Animals can often be relied upon to make the best decisions for their health and welfare in many situations. For example, when trained to distinguish between normal feed and feed containing carprofen, lame broiler hens would choose to consume the carprofen laced



feed, whereas the healthy hens would not. Furthermore, as the degree of lameness increased, the hens responded by increasing their intake of the carprofen feed [43].

## Practical Implications

Veterinary procedures can sometimes be negatively perceived by the animals involved, whether it's the result of handling by an unfamiliar person, fear from being socially isolated, or as a result of the pain experienced from the procedure [44-46]. Simple steps can be adopted to minimise the distress experienced by the animal. In the case of domesticated animals, gentle tactile contact has been shown to be effective in reducing distress. For example, in cattle, sheep and horses, gentle stroking and calm voices have been shown to reduce cortisol levels, heart rate, and flight distances during both veterinary procedures and handling [32,47-52]. In addition, the presence of a familiar and positively perceived person can have significant positive effects on the emotional experience of the animal [52]. Where possible, social isolation should be minimised, as this has been shown repeatedly in a number of species to be extremely stressful [32,33,45]. Simple steps such as these can have a considerable impact on the animals' experience. Furthermore, it can have positive effects on future interactions and make tasks easier to perform. Taking the animal's point of view can be a very helpful exercise when considering their mental well-being [41]. Animals are sentient, feeling beings, just like us, and their feelings matter to them and to us.

Veterinarians have a role in not only treating the animals they care for, but also in educating their owners in what is best for their animals. Emphasising the importance of considering the mental lives of animals is crucial as it is so often neglected, yet it has major implications for the health and welfare of animals. The links between poor mental health and physical health have been well documented [53]. There is now also a growing interest in the effects of positive experiences on the physical health of animals [54]. This is a burgeoning area of research in humans, and research is exploring whether laughter and positive experiences can have a positive effect on physical health [55,56]. In cattle, it was found that positive treatment of heifers resulted in subsequent improved parlour behaviour and milk production [57], and that farms where cows were called by name reported significantly higher milk yields than those where this was not the case [58]. Much more has to be done to further explore these effects in animals, but the overwhelming evidence for the relationship between negative emotions

and physical health gives a strong indication that there will be a significant link between positive emotions and health. Either way, given that animals are sentient, feeling beings, it is important to ensure that they experience positive feelings and emotions for the sake of their welfare, and any benefits to their health or levels of productivity should be seen as an additional benefit.

World Animal Protection is committed to promoting the science of animal sentience, and has developed a website which is dedicated to this area of science. The Sentience Mosaic is a great resource for veterinarians and more information can be found at [sentiencemosaic.org](http://sentiencemosaic.org) and in the article 'Animal Mosaic: Collaborating online for animal welfare' in this issue.

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~ Helen Proctor  
Sentience Manager  
World Animal Protection

## **Animal welfare: focusing on the future – An OIE Publication**



*Published by the World Organisation for Animal Health (OIE) in its Scientific and Technical Review 33 (1) 2014, this issue provides up-to-date and forward-looking perspectives on developments in animal welfare policy, science and practice. It contributes to the OIE Global Animal Welfare Initiative, endorsed by all 180 member-countries of the OIE, by introducing non-specialists to current concepts and likely future developments in animal welfare internationally. It will be of interest to animal-based scientists, including livestock scientists and veterinarians, tertiary-level educators and student, policy makers, animal welfare groups and others with an interest in improving the care, productivity and management of animals, especially livestock, in different parts of the world.*

There are two major themes. The first is that animal welfare is a complex, multifaceted, international and domestic public policy issue with scientific, ethical, economic, religious and cultural dimensions plus important trade policy implications. Although this theme is apparent throughout, it receives particular attention in the seven papers in *Section 1*, which illustrate in various ways the interactive character of these numerous drivers and the dominant ones in the five different OIE regions.

The second theme, apparent in all subsequent sections, is the importance of having secure scientific foundations as a basis for management practices that aim at maintaining animal welfare at acceptable levels.



*Section 2* firmly establishes this theme with three papers that explore the current status and future implications of new ideas about interactions between emotion and cognition in animals, the use of ethological and health indices of welfare, and how these and other factors help to provide validated scientific foundations for animal welfare standards. The pivotal importance of stockperson attitudes and skills in securing the welfare and productivity of farm livestock and working animals is then outlined in the paper in *Section 3*. This paper represents an appropriate introduction to the two in *Section 4*, which deals with the management of intensive livestock production systems, because the role of stockpersons is likely to assume greater significance in the future in view of the anticipated worldwide expansion of such production systems. These papers deal with the future application of lessons learnt in the past and with science-based management of livestock welfare in intensive systems.

The next four papers are relevant to both intensive production systems and pastoral/rangeland environments. The first two, in *Section 5*, deal with ways of improving the genetic 'fit' of animals with their environments. Thus, they consider key animal-environment compatibilities and incompatibilities that may guide the genetic selection of animals and/or the choice or manipulation of environments that may promote animal welfare. The two papers in *Section 6* consider how technology may be used to enhance welfare monitoring and management. Thus, they outline current and anticipated technological applications to rangeland livestock over long distances and multifaceted monitoring in intensive husbandry systems.

The remaining papers consider the welfare of particular classes of animals.

The first paper in *Section 7* deals with the welfare of working animals in Africa and Asia, and seeks to identify problems, consider solutions and anticipate future developments. The second paper focused on working equines in South America and emphasises strategies that are currently being deployed to improve their welfare.

Companion animal welfare is considered in two papers in *Section 8*. The first explores the impacts of breeding, behaviour and householder lifestyle on cat and dog welfare. The second analyses welfare-related issues that may arise with non-traditional pets, and based on that analysis provides a checklist for evaluating whether the welfare of such animals can be secured in home environments and, thus, whether or not they are suitable to be kept as pets.



The welfare of farmed fish is explored in *Section 9*, which includes three papers. The first introduces the concept of fish welfare and outlines how it may be assessed and promoted; the second discusses pain perception and the causes, and consequences of stress in fish; and the third outlines key elements of the humane harvesting and slaughter of fish. Taken together, these papers introduce this relatively new area of welfare evaluation, the significance of which will continue to increase as the anticipated rise in aquaculture to provide protein for the growing human population gains momentum.

Laboratory animal welfare is the focus of *Section 10*. The first of two papers presents the wide range of factors that should be considered when assessing the justification for particular laboratory studies by conducting harm-benefit analyses, and strongly emphasises the minimisation of harm. The second paper provides support for the proposition that an increase in biological variability potentially introduced by welfare-enhancing environmental enrichment initiatives improves, and does not undermine, the scientific validity of laboratory animal studies.

*Section 11* includes four papers on the disposal and management of pest and diseased animals. The first outlines an approach to ranking the humaneness of vertebrate pest control and culling methods; the second explores how humane slaughter house practices may be applied to large scale culling; the third evaluates humane killing of large numbers of animals for disease control purposes; and the fourth considers initiatives to control canine rabies in developing countries and some animal welfare implications of the methods used. A common theme in these papers is recognition of the potential for these activities to elicit negative emotional responses in those who undertake them, those whose animals are killed, and others who witness the events at a distance. A further theme is that the urgency of some mass killing events may justify use of less humane techniques than might otherwise be chosen, but that, nevertheless, the most humane methods available, given the full circumstances of the event, should be used.

Finally, the single paper in *Section 12* sums up and considers some possible future developments.

Publication of *Animal Welfare: Focusing on the Future* was instigated and its content conceived, refereed and edited by members of the five-partner OIE Collaborating Centre for Animal Welfare Science and Bioethical Analysis based in New Zealand and Australia (<http://www.oie.int/our-scientific-expertise/collaborating-centres/list-of-centres/>), with able support from OIE Paris staff and their associates.

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## Book & Journal Programme



Dr. Jeff Cave

The CVA Book Programme is coordinated by Dr. Jeff Cave

### CONTACTS:

Dr. Jeff Cave  
Department of Primary Industries  
1 McKoy Street Wodonga  
Victoria 3689, Australia  
E-mail: jeff.cave@dpi.vic.gov.au  
Tel: (66 0) 2 6043 7958 (O)

The CVA Book Programme is coordinated by Dr. Jeff Cave in Australia. Books are donated by veterinarians in Australia and New Zealand, all of whom are thanked for their generosity, without which the programme would not exist. They are available for distribution free of charge to graduate veterinarians in CVA member countries in good standing. Priority is given to requests from institutional libraries, such as veterinary schools and veterinary associations, and requests from individuals are met as funds permit. Postgraduate students are encouraged to submit their requests through the librarian at their institution, to ensure that the books

will be widely available. Because of budgetary constraints and steeply rising mailing costs, the number of books which can be shipped is normally restricted to up to 20 titles for institutions, and up to 5 titles for individual veterinarians in any one year. Individual veterinarians are encouraged to share their books with colleagues in their area if possible.

Requests for books should indicate the required subject areas and/or preferred titles where possible, and they should include the mailing address to which the books should be sent. The latter should be abbreviated as much as possible in order that it may be accommodated in the limited space provided on the customs declaration. It is suggested that those wishing to submit a request should first obtain a copy of the current inventories of books available by contacting, preferably by e-mail, Dr. Cave. Shipments are made by surface mail, and may take several months to reach their destination. The recipients are requested to acknowledge the safe arrival of the books.

During the period July 2013 - June 2014, from Australia and New Zealand, 234 books were sent to 14 different countries as follows: Zambia 78, Belize 43, Tanzania 21, Fiji 18, East Timor 15, Pakistan 12, Zimbabwe 11, Trinidad and Tobago 8, Papua New Guinea 7, Bangladesh 6, India 6, Malawi 5, South Africa 3 and Ghana 1.

The current inventory in Australia and New Zealand comprises nearly 350 books with over 250 different titles. Most of the books were published during the last 20 years; older texts, for which more recent editions are available, are discarded each year. Most areas of veterinary medicine are covered.

July 2013

JEFF CAVE  
Coordinator – CVA Book Programme

### 6th Pan Commonwealth Veterinary Conference

The 6th Pan Commonwealth Veterinary Conference of the Commonwealth Veterinary Association and 27th Conference of Veterinary Association of Malaysia (VAM) will be held from 23th – 27th March 2015 at Hotel Royale Chulan, Kuala Lumpur, Malaysia.

The theme of conference is “*Providing Holistic Solutions to Changing Global Challenges: Threats and Opportunities for Veterinarians*”.

The conference will cover topics including one health, neglected zoonoses, livestock health and welfare, food security, education, antimicrobial resistance and the human animal bond.

This conference will provide many opportunities to learn, share and network with acclaimed veterinarians from all Commonwealth and non Commonwealth countries of the world. The Conference is expected to attract more than 400 delegates.

The Scientific Programme which is being developed by the Scientific Committee under the Chairmanship of Prof. Dr. Mohd Hair Bin Bejo, Dean, and Faculty of Veterinary Medicine Universiti Putra Malaysia will include - International and Regional Speakers; Lectures, Plenary Sessions and Free Papers and Poster Presentations; and Pre- & Post-Congress Workshops in Livestock and Small Animal Medicine.





## CVA Study Fund

### The Fund

This fund has been established by the Commonwealth Veterinary Association (CVA) in conjunction with the Commonwealth Foundation to honour the contributions made by Mr. John Anderson and Dr. L.P.E. Choquette in establishing and promoting the activities of the Commonwealth Veterinary Association.

Financial support to match the funds contributed by the Commonwealth Veterinary Association and the several national and local veterinary associations throughout the Commonwealth may be provided by the Commonwealth Foundation.

### 1. Purpose

Its purpose is to provide financial assistance to:

1. Veterinarians who are members in good standing of their respective national associations to undertake short term study visits to schools, institutions or to undertake short term study courses in veterinary medicine, animal production or related areas in other Commonwealth countries.
2. Animal Health Assistants recommended by the appropriate CVA Council Member and Regional Representative, to undergo further short-term training at a school or institution in another Commonwealth country.

It is expected that such visits will promote professional and para-professional contacts and provide grantees with new knowledge and expertise in their respective fields of interest. Study proposals which will directly benefit the rural poor and disadvantaged will receive sympathetic consideration. All proposals will be expected to describe how they will benefit the home institution, veterinary organization and community. The visit is also expected to result in a broadening of cultural experience and horizons and to promote Commonwealth understanding.

### 2. Guidelines

1. Grants will be limited to persons with field experience and not holding senior positions.
2. The awards are not normally available for University academic or research staff.
3. Preference will be given to related regions with 'south-south' movements being encouraged. In exceptional cases, visits to institutions outside the regions qualifying under south-south arrangement will be considered as long as the cost of the visit does not exceed the allocated fund award (Aus \$ 3000). In exceptional circumstances and where approved by the President grantees may receive training in a non-Commonwealth country within that Region.
4. The study period should be preferably between 2-3 weeks.
5. Awards will normally be distributed equally amongst Regions, however, on occasion, the President may authorize additional awards to a particular Region in any one year.
6. The study visits will be financed at a maximum of Aus \$ 3000 including a prepaid air ticket for the least expensive and most direct route.

7. Grants are provided only for periods of concentrated study or training on a particular topic or activity and cannot be made for attendance at conferences, meetings etc., nor to underwrite a tour of visits to a number of institutions.
8. A report must be submitted to the Secretary CVA within three months of the completion of the study visit. At the completion of the study visit, the participant must receive a letter of release, which should clearly indicate duration of stay, and satisfactory completion of course. The letter should also confirm that at the time of departure, the participants have not left any debts unsettled. This requirement must be conveyed by the Regional Representative or Programme Director to the host institution before arrival of participant.
9. It will be necessary for the host institution to agree to assist in arranging suitable accommodation etc. affordable by the applicant.
10. Grantees will be expected to give one or two lectures at the host institution or veterinary association on aspects of animal health and production activities in their home country. These lectures should emphasize how their studies in the host country will benefit the rural poor and disadvantaged as well as their impact upon the environment.
11. These lectures and the discussions of topics, both professional and social, with the staff of the host institution or veterinary association will serve to further the aims and objectives of the Commonwealth Veterinary Association.

### 3. Applications

- i) There is a set Study Application Form/Application. Forms are available from the CVA Secretary, or through the CVA Website.
- ii) Applications should be submitted to the appropriate Regional Representative for processing, at least 6 months prior to the proposal visit.
- iii) The applicants should provide the following:
  - a) A complete curriculum vitae to the Regional Representative
  - b) Two passport size photographs
  - c) A letter of acceptance from the person who will supervise the study program in the host country
  - d) Evidence that the study has the support of his/her home institution or national association

### 4. Administration

- i) The Study Application Form with supporting documents must be sent to the appropriate Regional Representative
- ii) The Regional Representative will review the application and make a recommendation to the Secretary, CVA.
- iii) The Secretary, CVA will make a recommendation to the CVA President, who will make the final decision.
- iv) The Secretary, CVA will then inform the Regional Representative who will inform the candidate.

**Last date of submission of request to Council Members/Reg. Rep. is 30<sup>th</sup> Oct. 2014. RRs to submit their recommendations before 30<sup>th</sup> Nov. 2014 to the Secretary, CVA.**

## CVA Officers visit BVA

The Officers of CVA namely, Dr. S. Abdul Rahman, President; Dr. Karen Reed, Secretary; Dr. Peter Thornber, Treasurer; Dr. Bob McCracken, Programme Director met in London on 27th June 2014 and critically reviewed the plan of work of the CVA and the future programmes especially the CPD programme in Africa. They also discussed the financial situation of the CVA and the urgent need to augment funds through corporate sponsorship.



*L-R: Karen Reed (CVA Secretary), Dr S Abdul Rahman (CVA President), Robin Hargreaves (BVA President), Dr Peter Thornber (CVA Treasurer), Dr Bob McCracken (CVA Programme Director)*

Making use of the opportunity of being in London they called upon the President of British Veterinary Association Dr. Robin Hargreaves and briefed him about the activities of CVA and extended an invitation to him to participate in the PCVC-6 next year.

They also visited World Animal Protection (WAP) formerly WSPA and had detailed discussions with their executive on the role of WAP in CVA projects and programmes. WAP has been generous to offer support to PCVC-6.

During the visit of Dr. S. Abdul Rahman to Paris for the OIE Animal Welfare Working Group Meeting, he along with Dr. Peter Thornber, Treasurer CVA and Dr. David Bayvel (WAP) met Dr. Bernard Vallat, Director General of OIE and requested him to provide OIE support to PCVC-6 and also invited him to the conference which he has graciously accepted. We have since then received confirmation of the support of OIE to the Conference.

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## Progress towards Rabies being a Notifiable Disease in the State of Karnataka, India - An APCRI /GARC/CVA Initiative

An announcement at the 16th National Conference of Association of Prevention and Control of Rabies in India (APCRI) held on 5-6 July in Mysore, India demonstrated for the first time a political willingness to make rabies a notifiable disease in India.

Prior to inaugurating the Annual Conference of APCRI, the State Health and Family Welfare Minister U.T. Khader had a meeting with Dr. S. Abdul Rahman, President of CVA and APCRI and Country Head GARC, India, Dr. Mahendra, Chairman Organising Committee of APCRICON 2014, and Dr. Pushpa Sarkar, Director of Mandya Institute of Medical Sciences wherein the importance of making Rabies a notifiable disease was stressed by Dr. Abdul Rahman. Following this during his

inaugural address, the Minister made a commitment by saying "Rabies, like the malaria and polio diseases, must be made a notified disease by the Central Government in order to adopt preventive measures against rabies, which is highly infectious and severe, affecting domestic animals, wildlife conservation, public health and livestock economies"

A notified (or notifiable) disease is any disease that is required by law to be reported to government authorities. If rabies is declared as a notified disease, then it attracts various provisions of the notified disease regulation act which enlists various responsibilities on the part of the individual doctors, health workers and the community medicine people, specific authorities can be

held responsible for an outbreak, and there will be more awareness about it. Surveillance data can also be collected and analysed, resulting in better estimates of the number of cases and a more accurate evaluation of the rabies burden in an area. This can then inform decisions on rabies control efforts that can save human lives.



**Health Minister U.T. Khader is seen inaugurating APCRICON 2014.**

*L-R: Dr. S. Abdul Rahman, President of CVA & APCRI, Dr. Pushpa Sarkar, Director, Mandya Institute of Medical Sciences, Minister, U.T. Khader, Dr. B.J. Mahendra, Organising Chairman, Dr. M.K. Sudarshan, Founder President of APCRI and Dr. M. Vinay, Organising Secretary.*

*Photo: Star of Mysore*

The minister said he intends to declare rabies as a "notified disease" in Karnataka State (in the South of India) and urge the union government to follow suit across India. Dr Abdul Rahman, will meet personally with him soon to pursue this further.

Minister Khader also called for prevention rather than cure and more awareness in schools on the risks of rabies. "Most of the people, especially the children's exposure to dog bites happen in the areas where they have no awareness about the severity of the dog bites and their fatality" he said, adding that awareness among the parents and people to protect their children from unwarranted dog bites will play a great role in reducing the incidents of rabies in the country.

"In case of the occurrence of such diseases, we blame the animals. But we humans are to be blamed because

we need to keep our environment clean and healthy" he pointed out and highlighted that citizens, animal lovers and NGOs need to take a pro-active role in rabies control instead of holding government responsible for everything. Minister Khader said that India can be rabies free if all join hands and work together. "It is a preventable disease. Conferences like

this should come out with suggestions and recommendations on eradicating rabies," he stated.

GARC and CVA had earlier initiated a project on Rabies being a notifiable disease in India by writing to all members of the Indian Parliament. Though there was a sympathetic response the then Union Minister of Health had replied that Rabies was a reportable disease which is not the same. Efforts were on at every Rabies forum to pursue this and this is the first time that a commitment has been given by a Health Minister of any state in India.

APCRI, CVA and GARC India will follow this up with the Government of Karnataka through Minister Khader and if it is made notifiable in Karnataka then it will be easy to cite the example and make it notifiable in other states of India and thus make India a country where Rabies will be notifiable law.

## Lab Animal Training Workshop in Pakistan

A two-days Laboratory Animal Training Workshop was arranged by Department of Clinical Medicine and Surgery at PGMI, Lahore during January 29-30, 2014.

Dr. Aneela Zameer Durrani, Chairperson, Department of Clinical Medicine and Surgery, Country Councillor, CVA made all necessary arrangements for this workshop in collaboration with PGMI. Dr. Muhammad Avais, Assistant Professor, Department of Clinical Medicine and Surgery, was the resource person for this two days training workshop and was assisted by Dr. Ghazanfar Ali Chishty, Lecturer Department of Clinical Medicine and Surgery.

More than 30 Faculty Members and Postgraduate students from Departments of Pharmacology, Anatomy

and Physiology participated in the workshop. Dr. Sadia Chiragh, Chairperson, Department of Pharmacology was also among the potential participants of the workshop.

Workshop was conducted in very conducive and learning environment on both the days. On Day-1, one hour lecture on Housing, Feeding, Management and Health care of Rabbit and Guinea Pig was delivered by Dr. Muhammad Avais. Lecture mode was two way, it was very interactive and participants asked different questions regarding problems faced by them during their research on lab animals. Dr. Muhammad Avais responded very well and nice clarified the queries. After that one hour hands on training session was conducted on handling and injection of rabbits and Guinea pig. Dr. Avais



*Dr. Muhammad Avais delivering lecture*



*Training session on handling of rabbits and Guinea pig*



*Demonstration of different methods on stress-free handling of rabbits and guinea pigs with various injection techniques and blood sampling methods*



demonstrated different methods of stress-free handling of rabbits and guinea pigs along with various injection techniques and blood sampling methods in these animals. On Day-2, same sequence of events was carried out but animals were rats and mice. It was also very interesting day with full of learning. At the end, participants individually practiced all the techniques on rabbits, guinea pigs, rats and mice.

In the last, Certificate Distribution Ceremony was arranged and Principal, PGMI, distributed certificates

among the participants. In concluding remarks, the participants showed their gratitude for UVAS team in solving the issues related to laboratory animals on scientific basis. Prof. Dr. Sadia and Principal, PGMI, paid their special thanks to Dr. Avais for his valuable time and dedication to for the successful conduct of the workshop. Dr. Avais assured them for every possible help from UVAS in future. In recognition, Principal PGMI awarded Dr. Muhammad Avais with appreciation shield and certificate.

~ Dr. Aneela Zameer Durrani  
CVA Councillor, Pakistan



## Caribbean Veterinary Medical Association 28th Biennial Conference

**November 4-7, 2014**  
**Grand Cayman Marriott Beach Resort**

Conference Update  
[www.CBVMA.org](http://www.CBVMA.org)



**“Opening New Frontiers in Veterinary Medicine: The Work, The Life, The Balance”**

### Hotel Rooms are Filling Fast!

Please use this link for information and reservations: <http://www.cbvma.org/hotel.html>

If you have troubles booking the room (incorrect rate, no rooms available, etc) please call this phone number for assistance: 1 345 949 0088, ext: 5703 or 5701

### Practical Labs

Practical Labs are brand new this year and are offered at a special discount for CbVMA attendees.

Two Practical Labs will be held on Friday, November 7, 2014.

**Slots will fill fast—register today!**

*Price includes all supplies, transportation, 1 hour pre-lecture and 3 hour hands-on lab session.*

- Discounted rates: our labs are less expensive than other conferences.
- Practical labs are three hours long and will be held on Friday 1:40-4:40 p.m.
- Labs are held off-site. Transportation from the Marriott and return is included in lab fee.
- Space is limited; be sure to register early to ensure your selection.
- Veterinarians and veterinary students may register for any practical lab.

#### ***Examination and Manipulation of Reproductive Tracts from the Slaughterhouse Including Suturing of the Uterus***

**Instructor: Maarten Drost, DVM, Dipl ACT**

3 hour lab; \$160

The laboratory exercises will first be illustrated with the Bovine Reproduction Guide of “The Drost Project” (<http://drostproject.org/>)

Next, slaughterhouse reproductive tracts will be used to 1.) palpate pregnant uteri under a plastic bag to block visual assessment; 2.) ultrasound tracts in water baths for signs of pregnancy and ovarian activity; 3.) suture uteri with the Utrecht method. Each attendee should plan to attend the morning lecture series.



#### ***Ultrasound Practical Lab***

**Instructor: Brian Poteet, DVM, DACVR, DABSNM**

3 hour lab; \$250

During this interactive lab session, participants will scan live canines using state of the art ultrasound machines (various brands of machines). The lab will assume a “beginner level” of the attendees but will quickly escalate in shared information. Finding and recognizing all normal abdominal organs will be emphasized. Any pathology found will also be shared with all participants. Each participant will scan for a timed period within a group, and then rotate to the next station. There will be a maximum of 6 stations, with no more than 6 participants per group. Instructors will supervise all scanning as well as share “tips and tricks” on how to find all of the abdominal organs and recognition of normal anatomy. Each attendee should plan to attend the morning lecture series.

### Registration is Open

**Register by August 31 and save money!**  
**Discounted rates for CbVMA members.**

**Click here for information and to register:** <http://www.cbvma.org/conference.html>

# CALENDAR OF EVENTS

## 2014

39th Annual World Small Animal Veterinary Association (WSAVA) Congress, Cape Town, South Africa.

**16-19 September**

25th International Rabies in the Americas (RITA) Meeting, Cancun, Mexico. **26-30 October**

IDF World Dairy Summit, Tel Aviv, Isreal. **27-31 October**

Aviana Uganda International Expo for Poultry and Livestock, Kampala, Uganda. **30-31 October**

28th Biennial Caribbean Veterinary Medical Association (CbVMA) Conference, Grand Cayman, Cayman Islands. **4-7 November**

8th Federation of Asian Veterinary Associations (FAVA) Congress, Singapore. **28-30 November 2014**

## 2015

6th Pan Commonwealth Veterinary Conference and 27th Conference of Veterinary Association of Malaysia (VAM), Kuala Lumpur, Malaysia. **23-27 March**

32nd World Veterinary Congress, Istanbul, Turkey. **13-16 September**

## 2016

Fourth OIE Global Conference on Animal Welfare, Chile. (Date and Venue to be announced)

## 2017

2017 World Veterinary Congress, Incheon, Korea. **August** (Dates to be announced)

## ADVERTISEMENT TARIFF

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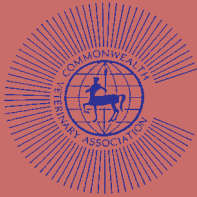
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## PRESIDENT

**Dr. S. Abdul Rahman**

123, 7th 'B' Main Road, 4th Block (West)

Jayanagar, Bangalore 560 011, India

Tel/Fax: (91 80) 26635210

Email: shireencva@gmail.com

## SECRETARY

**Dr. Karen Reed**

Head of Animal Welfare and Research

The Brooke, 30, Farringdon Street

London EC4A 4HH, UNITED KINGDOM

Tel: (44 0) 20 76535837; Fax: (44 0) 20 30120156

Mob: (44 0) 77 87130218

Email: Karen.Reed@thebrooke.org

## TREASURER

**Dr. Peter Thornber**

Manager, AAWS and Communications

Agricultural Productivity Division, DAFF

GPO Box 858, Canberra ACT 2601, AUSTRALIA

Tel: 02-6272-3925, Mob. 0407207986

Email: Peter.Thornber@daff.gov.au

## IMMEDIATE PAST PRESIDENT

**Dr. Richard D Suu-Ire**

PO Box 143, Legon, GHANA.

Tel: (233 21) 782177 (R); 772553 (O)

Fax: (233 21) 776021

Email: suuire@hotmail.com

## PROGRAMME DIRECTOR

**Dr. Robert McCracken CBE**

The Old Gallery, 1 Milford Road, Duffield, Belper

Derbyshire DE56 4EL, UNITED KINGDOM

Tel: (44 0) 1332 843130

Mob: (44 0) 7766355 465

Email: hbmccracken@btinternet.com

## REGIONAL REPRESENTATIVES

### ASIA

**Dr. A. Sivasothy**

Sri Lanka Veterinary Association, No.275/4

OPA Building, Prof. Wijesundara Mawatha

Colombo 07, SRI LANKA

Tel: (94) 71 8047701; 8081059

Fax: (94) 71 2389136

Email: casiva82@yahoo.com

### EAST/CENTRAL/SOUTHERN AFRICA

**Dr. Henry Magwisha**

Central Veterinary Laboratory, Mandela Road

Temeke Veterinary, PO Box 9254

Dar-es-Salaam, TANZANIA

Tel: (255 22) 2861152; Fax: (255) 22 2864369

Email: hbudodi@yahoo.com

### AUSTRALASIA/OCEANIA

**Dr. Paul Chelliah**

Veterinary Association of Malaysia

1800A, Jalan Tok Ungku 70100

Seremban, Negeri Sembilar

MALAYSIA

Email: pauveni@gmail.com

### WEST AFRICA

**Dr. Sulayman Sonko**

Gambian Veterinary Association

PMB 14, Banjul

THE GAMBIA, West Africa

Tel/Fax: (220) 392173

Email: sonko\_sulayman@yahoo.com

### CANADA/CARIBBEAN

**Dr. Curtis Padilla**

29, Pro Queen Street, Arima

TRINIDAD, West Indies

Tel: (868) 3287195; Fax: (868) 6640878

Email: easternvetclinic@yahoo.com

### UK/MEDITERRANEAN

**Dr. Karen Reed**

The Brooke, 30, Farringdon Street

London EC4A 4HH, UNITED KINGDOM

Tel: (44 0) 20 76535837; Fax: (44 0) 20 30120156

Mob: (44 0) 77 87130218

Email: Karen.Reed@thebrooke.org