ESI⁺ MS/MS confirmation of canine ivermectin toxicity†

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Ivermectin is a semisynthetic macrocyclic lactone anthelmintic of the avermectin family derived from Streptomyces fermentation products. Avermectins are used as antiparasitic agents in domestic animals; although considered relatively safe, one must consider animal species, breed, weight, and age in dosage determinations.

In January 2006, two canines were presented to the UK Livestock Disease Diagnostic Center after dying from suspected ivermectin overdoses (30–50 mg/kg body weight). To confirm this clinical diagnosis we developed a rapid, sensitive semiquantitative ElectroSpray Ionization–Mass Spectrometry (ESI/MS) method for ivermectin in canine tissue samples. Pharmaceutical ivermectin contains two ivermectins differing by a single methyl group, and each compound forms ion adducts with tissue Na⁺ and K⁺ ions. We now report that ivermectin administration was clearly confirmed by comparison with standard and dosage forms of ivermectin, and simple proportionality based on mass spectral intensity of respective molecular ions allowed semiquantitative estimates of injection site tissue concentrations of 20 and 40 µg/g tissue (wet weight) in these animals, consistent with the history of ivermectin administration and the clinical signs observed.

There is a distinct need for both rapid detection and confirmation of toxic exposures in veterinary diagnostics, whether for interpretation of clinical cases antemortem or for forensic reasons postmortem. It is vital that interpreters of analytical results have appropriate guidance in the scientific literature and elsewhere so as to enable clear-cut answers. The method presented here is suitable for routine diagnostic work in that it allows rapid extraction of ivermectin from tissue samples, avoids the need for high-performance liquid chromatography and allows ready interpretation of the multiple ivermectin species seen by ESI⁺ MS/MS in samples originating from veterinary dosage forms. Copyright © 2008 John Wiley & Sons, Ltd.

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Introduction

Ivermectin is a high molecular weight (MW 860–874) macrocyclic lactone anthelmintic, a member of the avermectin family of compounds derived from the fermentation byproducts of certain Streptomyces bacteria. It comprises 22,23-dihydroavermectin B₁a (≈80%) and 22,23-dihydroavermectin B₁b (<20%) according to Gfeller and Messonnier,11 herein referred to as ivermectin B₁a and ivermectin B₁b, respectively. Ivermectin was introduced in 1981 as an antiparasitic medication, and was rapidly accepted worldwide2,3; commercial applications in small animal medicine include Heartguard and Milbemax. Ivermectins are effective against many endo- and ectoparasites such as intestinal worms, mites and lice, and are used for prevention of heartworms in dogs and cats, and hookworms in cats. Ivermectins can be administered by many routes with due consideration for animal species type, weight, age and – in some cases – breed of the animal patient.1,4

Avermectins are agonists for the neurotransmitter gamma-aminobutyric acid (GABA), a major inhibitory neurotransmitter. In mammals, GABA-containing neurons and receptors are found in the central nervous system (CNS).5 In contrast, arthropods and nematodes retain GABA-receptors primarily in the peripheral nervous system (PNS), specifically in the neuromuscular junction (NMJ). This difference in location is the primary reason that ivermectin-containing products are selectively toxic to arthropods and nematodes, yet safe for use in mammals. Binding of ivermectin to neuronal membranes increases the release of GABA, increasing GABA receptor-chloride channel complexes of postsynaptic neuronal membranes, thereby causing an influx of chloride ions.1,5 This in turn hyperpolarizes the neuronal membranes, makes them less excitatory and decreases nerve transmission. Hyperpolarization of neuronal membranes at the NMJ thereby mediates paralysis in arthropods and nematodes.5

Avermectins are effectively excluded from the CNS of vertebrates by a P-glycoprotein-mediated efflux mechanism.1,5 However, certain breeds of dogs, such as Collies, Shetland and English sheepdogs and Australian shepherds are deficient in this
P-glycoprotein, and are therefore much less effective at preventing avermectins from crossing the blood-brain barrier. As a result, Collie-type dogs, for example, are highly susceptible to avermectin toxicosis.[6,7] The American Board of Veterinary Toxicology (ABVT; www.abvt.org) reports the following doses of ivermectin as sufficient to induce clinical signs of intoxication in canines: 0.1–0.2 mg/kg in Collies (15–30 times the therapeutic dose) in contrast with 2.5–40 mg/kg in beagles (>200 times the therapeutic dose).[8–11] Intoxication is characterized by mydriasis, depression, coma, tremors, ataxia, emesis, stupor, hypersalivation, and death.[7,12]

Mass spectrometric techniques have been elaborated for visualization or confirmation of ivermectins and other macrocyclic lactones (ML). Danaher et al.[13] have provided a comprehensive review of ML antiparasitic agents including ivermectin, their range of activities and uses in food-producing animals and crops, methods for extraction from various matrices and derivatization for spectrometric analysis. They conclude that suitable determination of MLs would use ion trap instruments with atmospheric pressure chemical ionization (APCI) detection of [M – H]− and [M + Na] + ions in negative or positive modes, respectively, or [M + H] + or [M + Na] + with ESI+ LC-MS/MS. For example, Hernando et al.[14] have recently reported a rapid chromatographic LC-MS/MS method using multiple reaction monitoring applicable to the MLs abamectin, ivermectin, emamectin benzoate and doramectin.

Extending this approach, we now describe a simple extraction procedure applicable to necropsy tissues for the rapid semiquantitative identification of ivermectin compounds in support of a diagnosis of ivermectin toxicity.

**Experimental**

**Compounds**

A 1-mg/ml ivermectin (Sigma–Aldrich, St. Louis, MO) stock solution was prepared in ethyl alcohol (EM Science, Gibbstown, NJ). The ivermectin ESI-MS-MS standard was a 1:50 dilution of the stock solution prepared by adding 40 µl to 1960 µl of ethyl alcohol.

Ivomec (Merial, Whitehouse Station, NJ) is marketed as a 1% sterile injectable solution.

**Animals**

In January 2006, two dogs were presented to the University of Kentucky, Livestock Disease Diagnostic Center, after dying from a suspected ivermectin overdose. A 1-yr-old, 5.5 lb (2.5 kg) male Shih Tzu (C998-06) and a 4-yr-old, 9 lb (4.1 kg) female Pekinese (C999-06) were each reportedly treated with 12.0 cc ivermectin (Millipore Corp, Bedford, MA) into a labeled brown vial. Filtration provided approximately 0.5 ml of each sample, to which 0.5 ml of 0.1% formic acid was added.

**Sample preparation**

Samples C998-06 and C999-06 were prepared for direct infusion ESI (positive mode) MS analysis by dilution to 1:10 with 0.1% formic acid (aq):acetonitrile, 1:1 mixture. Extracts were treated similarly, with the exception that ethyl alcohol was substituted for acetonitrile since acetonitrile was found to induce cloudiness in the extract under these conditions. Infusion was carried out with a syringe pump (Harvard Apparatus, Holliston, MA) equipped with a 500 µl Hamilton (Hamilton Co., Reno, NV) gastight syringe with infusion at 1.5 ml/h.

The mass spectrometer was a Micromass (Waters, Inc., Beverly, MA) Quattro II ESI-MS/MS electrospray triple stage quadrupole (TSQ), and typical ESI-MS voltage settings for detection and analysis were as follows: capillary, 3.22 kV; HV lens, 0.5 V; cone, 30 V; skimmer lens, 1.4 V; RF lens, 0.0; source temperature, 150 °C; argon pressure for collisionally induced dissociation (CID) experiments, 2 × 10–4 mbar; ionization energies: MS1, 0.2 V; MS2, 3.9 V. Collision energy was set between 30 and 60 eV. ESI-MS and MS/MS spectra were acquired as continuum data for a minimum of 1–2 min over the m/z 10–1000 mass range, applying 3.1 s per scan duration. Resultant data were background-subtracted and smoothed with the Micromass MassLynx version 3.4 software. Spectra were deconvoluted with the assistance of Mass Spec Calculator Pro software, version 4.03.[15]

Ivermectin spectra were also acquired with a Thermo LC/MS (Thermo–Fisher Scientific, Waltham, MA) equipped with ESI and an ion trap detector. The system was carefully calibrated with polyethylene glycol 400 standard and then the accuracy assessed by successful comparison to published high resolution data for styrchnine m/z 206.0982, 234.0935, 246.0938, 264.1014, 272.1064, 290.1169, 307.1434 and 335.1754 fragments[16] to determine <1000 ppm error in ivermectin fragment molecular weight measurements by ESI-low resolution MS.

Accurate mass CID spectra were generated using a QTOF Ultima mass spectrometer (Waters Corp., Milford, MA) operated using positive mode electrospray ionization and flow injection analysis. Mass calibration was performed using a mixture of polyethylene glycol (PEG) 300 and 600, and mass accuracy was optimized using a LockSpray source attachment, switching between CID spectra of sample ions and CID of [M + NH4] + ions from the PEG reference solution.

**Hyperchem molecular modeling**

A structural model of ivermectin B1a was constructed with the Hyperchem release 3 (1993) molecular modeling program (Hypercube, Inc., Gainesville, FL). The stereochemistry was checked and adjusted for accuracy, after which an initial structure was calculated with Hyperchem molecular mechanics using the MM+ force field. Geometry and electronics were then optimized by application of the AM1 semiempirical method using the Polak–Ribiere algorithm. Calculated heteroatom (i.e. oxygen atom) partial charges were considered to impart information on local electronegativity, in line with theories of Allred and Rochow.[17]
Results

Clinical history

Within 7 h of purported treatment with 12.0 cc 1% ivermectin, each of the dogs showed clinical signs consistent with ivermectin toxicity and was taken to a licensed veterinarian (JW Seantor) for treatment. The veterinary report listed that the Shih Tzu was in cardiac arrest and in a comatose state upon arrival. The Pekinese had dilated pupils and ataxia. Emergency therapy was unsuccessfully attempted on both animals.

Skin scrapings were taken from each animal to confirm whether mange mites were present. Results were negative. The final veterinary report listed that visible skin lesions were not consistent with mange, and furthermore the condition of the skin more closely resembled that of an allergic reaction or a severe skin infection.

Necropsy reports

Gross examination of the specimen C998-06 revealed the canine’s hair to be sparse, retained over the neck, shoulders and back. There was little hair on the sides, abdomen and legs of the animal. The pathology report noted the skin to be irregular, thickened and red with multiple lesions up to 1.0 cm in size and covered by brown scabs. Behind the left shoulder and over the thorax, the most likely site of injection, the subcutis was exposed and found to be glistening and slightly discolored pale green. The specimen C999-06 was submitted with no visible lesions, except for a 0.5 cm bruised area in the subcutis over the dorsum of the right thorax, the site of the suspected injection of ivermectin. Gross examination here also revealed the subcutis to glisten with small pockets of clear liquid within the deep fascia of the ventral abdomen. On comparison of the two cases, the subcutis in both animals glistened and was slightly discolored over the dorsum, behind the shoulders at the likely site of injection as shown in Fig. 1.

Analytical chemistry

The structure of ivermectin (Fig. 2) reveals that both B1a and B1b forms are 16-member macrolides with disaccharide substituents. Ivermectin typically consists of 80% B1a and 20% B1b, ivermectin analogs.[18] Although dissolved in our regular direct infusion electrospray solvent (acetonitrile:0.01% formic acid, aq, 1 : 1), ivermectin standard from Sigma studied by ESI-MS revealed a principal peak at m/z 897 rather than at an anticipated m/z 875 [M + H]^+ for the B1a analog peak, similar to results shown for the pharmaceutical dosage form in Fig. 3 (left). This was rationalized as being the result of sodium ion complexation, with one possible route involving polarization of the ivermectin ester linkage into positive and negative charges at the double bond oxygen, as shown schematically in Fig. 2, lower right. This is presumably due in turn to sodium inextricably contained in the standard or in the injectable formulation.

Precedent exists for alkali metal ion binding to hydroxyl, ester and ether groups, particularly in sugars, as revealed by mass spectrometric or nuclear magnetic resonance techniques.[19–23] Hyperchem molecular modeling revealed a distribution of partial negative charges principally on oxygen atoms in uncharged ground state ivermectin B1a, ranging from −0.073 to −0.333. The inset figure to Fig. 2 indicates partial negative charges on oxygen atoms in excess of −0.300, and if we hypothesize that these are the preferred sites for alkali cation binding, then sodium or potassium could conceivably be bound at disaccharide, ester, spiro carbon ether, or hexahydrobenzofuran hydroxyl groups.

Although largely absent in the Sigma standard (Fig. 3, lower right), additional peaks were revealed in the diluted veterinary dosage form of ivermectin, particularly in clusters at m/z 883, 886, 894 and 913, as shown in Fig. 3 (left). Most of the peaks were interpreted by inclusion of potassium ions as possible complexing agents as shown schematically in Fig. 2 (right) with consideration of both principal forms B1a and B1b, providing m/z 883, 897, 899 (overlaps m/z 897 isotopic M + 2 peak) and 913.

The additional peaks seen in the veterinary preparation and providing clusters at m/z 886 and 894 were unusual in that isotopic abundance peaks increased by 0.5 rather than 1.0 unit mw. This suggested doubly charged species, and calculations revealed the simplest interpretation as ivermectin B1a dimers complexed with both a proton and a sodium ion (m/z 886) or with a proton and a potassium ion (m/z 894). Table 1 shows the content of these species in the pharmaceutical preparation (column 2), where these numbers assume a direct relationship of mass spectral abundance with percent composition; the combined results suggest 95–96% ivermectin B1a and 4–5% B1b in this pharmaceutical preparation. Corresponding peak assignments are listed in the final column. Results for canine samples in columns 3 and 4 indicate comparability between ivermectin populations in the pharmaceutical prep with those in the animal samples. Expectations of greater availability of potassium in tissue extracts and lower overall concentration of ivermectin explains the increase in potassium adducts and decrease in dimers.

Table 2 examines isotopic peaks adjacent to the prominent mass spectral species m/z 897 and 913 in Fig. 3 visualized in the pharmaceutical formulation. There is reasonable comparability between the measured and theoretical values for the M + 1, +2 and +3 isotope abundances of the most intense peak at m/z 897; note that a slightly high M + 2 peak can be rationalized as arising from an otherwise invisible ivermectin B1a + K^+ complex calculated at m/z 899. The m/z 913 potassium complex similarly shows excellent

![Figure 1. Subcutaneous tissue removed from between the shoulder blades of C998-06, showing the glistening pale green discoloration at the site of ivermectin injection.](image-url)
Ivermectin \( B_{1a} \) and \( B_{1b} \) structures are disaccharide-substituted 16-member macrocyclic lactones differing by a methylene group. Ivermectin \( B_{1a} \) is \( \text{mw} = 874 \), while \( B_{1b} \) is \( \text{mw} = 860 \). The reaction scheme to the right indicates hypothetical charge separation at the lactone ester linkage (in brackets), with subsequent coordination of a potassium (upper right) or sodium (lower right) to give positively charged species of the molecular weights indicated. The inset figure shows Hyperchem-calculated partial negative charges in excess of \(-0.300\), suggesting that there are multiple locations where alkali cations could bind.

Figure 2. ESI-MS(+) full scan examination of ivermectin pharmaceutical form (1.0%) diluted 1:100 in 0.1% formic acid: acetonitrile, 1:1, and infused at 1.5 ml/h (left). Peak labels show measured \( m/z \) value (upper value) and measured intensity (lower value). Examination of C998-06 tissue ethanol extract acidified 1:1 with 0.1% formic acid is shown (top right) in comparison to a Sigma ivermectin standard prepared similarly (bottom right).

Figure 3. ESI-MS(+) full scan examination of ivermectin pharmaceutical form (1.0%) diluted 1:100 in 0.1% formic acid: acetonitrile, 1:1, and infused at 1.5 ml/h (left). Peak labels show measured \( m/z \) value (upper value) and measured intensity (lower value). Examination of C998-06 tissue ethanol extract acidified 1:1 with 0.1% formic acid is shown (top right) in comparison to a Sigma ivermectin standard prepared similarly (bottom right).
comparability between the measured and theoretical values for M+1, +2 and +3 isotopic abundances. The B1b complex at /z/883 and the dimers at 886 and 894 show inferior agreement between the measured and theoretical values, particularly for M + 2 and M + 3 isotopes, which could be justified on the basis that these peaks are only <10% of the major /z/897 B1s sodium adduct, and for the dimers by the fact that the isotopic peaks do not show clear baseline resolution.

High resolution mass spectrometry confirmed the identities of the principal ivermectin B1s components at /z/897 and 913 by virtue of their accurate masses of 897.4973 and 913.4741, respectively. These corresponded in turn to C48H74O14Na (calculated 897.4977 mw) and C48H74O14K (calculated 913.4716 mw), respectively, within tolerances of less than 5.0 ppm difference.

Table 2. Isotopic abundance analysis of ivermectin species; theoretical values were calculated with the assumption of correct identity assignments in Table 1

<table>
<thead>
<tr>
<th>M, /z/</th>
<th>M + 1, measured (theoretical) (%)</th>
<th>M + 2, measured (theoretical) (%)</th>
<th>M + 3, measured (theoretical) (%)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>883</td>
<td>61 (54)</td>
<td>30 (17)</td>
<td>74 (3)</td>
<td>M + 3 increased by overlapping /z/886</td>
</tr>
<tr>
<td>886a</td>
<td>117 (110)</td>
<td>115 (66)</td>
<td>78 (27)</td>
<td></td>
</tr>
<tr>
<td>894a</td>
<td>95 (110)</td>
<td>78 (66)</td>
<td>28 (32)</td>
<td></td>
</tr>
<tr>
<td>897</td>
<td>54 (55)</td>
<td>21 (18)</td>
<td>5 (4)</td>
<td>/z/897 increased by B1b + K+</td>
</tr>
<tr>
<td>913</td>
<td>55 (55)</td>
<td>28 (25)</td>
<td>10 (8)</td>
<td></td>
</tr>
</tbody>
</table>

* Doubly charged dimers; values for these increase by 0.5 rather than 1 unit, thus M + 0.5, M + 1, M + 1.5.

Discussion

In this report, two canines were reportedly administered ivermectin by the subcutaneous route for treatment of mange mites and died shortly after administration of the pharmaceutical agent; the user had apparently overlooked possible significant advantages to pour-on, drench or oral formulations. The history and the user had apparently overlooked possible significant advantages to pour-on, drench or oral formulations. The history and clinical signs led to the suspicion that the dogs suffered from ivermectin toxicity. Although most modern anthelmintics have a wide range of safety,[6] increase of the dosage rate of the medication corresponds to a decrease in its safety margin.[24,25]

The focus of our work was to develop a direct infusion, electrospray ionization/tandem mass spectrometry (DI-ESI-MS/MS)
Figure 4. ESI-MS daughter ion spectrum (left) of ivermectin dosage form (= 1.0% ivermectin) diluted 1:100 in 0.1% formic acid: acetonitrile, 1:1 mixture and infused at 1.5 ml/h. Examination was made specifically of fragments of the principal peak at m/z 897, for which the collision energy setting was 45 V. Note that cases C998-06 and C999-06 described in the text provided convincing matches to this pattern of mass spectral peaks. The accompanying schematic shows the origin of principal fragments from ivermectin B₁₃-sodium complex (mw 897) as supported by high resolution MS data (see Table 3).
Table 3. Comparison of ESI-TSQ (Quattro II), ESI-ion trap (Thermo), and ESI-high resolution (QTOF) m/z values for principal ivermectin B1a m/z 897 daughter ions, including derived molecular formulae for each peak

<table>
<thead>
<tr>
<th>ESI-TSQ value</th>
<th>ESI-ion trap value</th>
<th>High RES QTOF MS</th>
<th>ESI-ion trap MS, ppm difference</th>
<th>High RES QTOF MS, ppm difference</th>
<th>Molecular formula</th>
<th>Calculated mw</th>
</tr>
</thead>
<tbody>
<tr>
<td>183</td>
<td>182.8</td>
<td>183.0639</td>
<td>-1378.2</td>
<td>3.28</td>
<td>C_7H_{12}O_4Na</td>
<td>183.0633</td>
</tr>
<tr>
<td>329</td>
<td>329.0</td>
<td>329.1779</td>
<td>-384.9</td>
<td>61.37</td>
<td>C_{14}H_{26}O_7Na</td>
<td>329.1577</td>
</tr>
<tr>
<td>449</td>
<td>449.2</td>
<td>449.2530</td>
<td>-37.8</td>
<td>-2.23</td>
<td>C_{25}H_{37}Na</td>
<td>449.2540</td>
</tr>
<tr>
<td>457</td>
<td>457.1</td>
<td>457.2225</td>
<td>-254.1</td>
<td>5.03</td>
<td>C_{24}H_{34}O_7Na</td>
<td>457.2202</td>
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<tr>
<td>497</td>
<td>497.3</td>
<td>497.2522</td>
<td>97.3</td>
<td>1.21</td>
<td>C_{25}H_{37}Na</td>
<td>497.2516</td>
</tr>
<tr>
<td>609</td>
<td>609.3</td>
<td>609.3369</td>
<td>-0.5</td>
<td>-5.58</td>
<td>C_{34}H_{50}O_8Na</td>
<td>609.3403</td>
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<tr>
<td>753</td>
<td>753.5</td>
<td>753.4199</td>
<td>74.2</td>
<td>1.06</td>
<td>C_{41}H_{62}O_11Na</td>
<td>753.4191</td>
</tr>
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</table>

Figure 5. ESI-MS daughter ion spectra of m/z 897 peak in ivermectin standard (top) in comparison to that in case 998-06 tissue extract (bottom). These spectra were captured at a collision energy setting of 60 V (higher in Fig. 4) to enable greater spectral fine structure at the lower masses. The ‘fingerprint’ match essentially provides conclusive confirmation of ivermectin-B1a in the sample.

Table 4. Ivermectin concentration in relation to weight of the animal

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Tissue concentration (µg/g)</th>
<th>Presumed dose (12 cc × 1%/kg)</th>
<th>Percentage of dose detected, (µg/g x g tissue)/total dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>C998-06</td>
<td>48</td>
<td>48</td>
<td>0.015%</td>
</tr>
<tr>
<td>C999-06</td>
<td>36</td>
<td>29</td>
<td>0.030%</td>
</tr>
</tbody>
</table>

a Ivermectin composition was estimated in samples by reference to a 10 µg/ml standard run under the same conditions and by assuming the following relationship where

\[ l = \text{background-subtracted mass spectral intensity} \]

\[ \text{Sample concentration} = \frac{[\text{ivermectin B1a}] + [\text{ivermectin B1a} + \text{Na}]}{[\text{ivermectin B1a}] + [\text{ivermectin B1a} + \text{K}]} \]

\[ \text{Standard concentration} = \frac{[\text{ivermectin B1a}] + [\text{ivermectin B1a} + \text{Na}]}{[\text{ivermectin B1a}] + [\text{ivermectin B1a} + \text{K}]} \]

A reasonably efficient extraction method was first developed by reference to the literature. Meiser et al.\[26\] developed GC/MS and HPLC procedures for levamisole and ivermectin detection and Pozo et al.\[27\] developed LC-ESI-MS-MS methods to detect abamectin; both these groups had success using acetonitrile extractions. Kolar et al.\[28\] also used acetonitrile to extract avermectins from sheep feces for HPLC.

However, acetonitrile is incompatible with the syringe filtration apparatus similar to the centrifugal filtration used successfully in the past;\[29\] we therefore reviewed other possible solvents such as methanol;\[30,31\] and 5-hydroxy-1,3-dioxane: 4-hydroxymethyl-1,3-dioxolane, 3:2 mixture;\[32\] and settled on ethyl alcohol as a simple water miscible alcohol with eluotropic strength between that of methanol and acetonitrile.\[33,34\]

The ESI-MS-MS analytical method, using simple ethyl alcohol extraction, yielded good results with many advantages. Equipment requirements are minimal, so the method is simple and efficient, since syringe filtration restricts the analysis to compounds of less
than ~3000 mw. This reliable and sensitive ESI-MS-MS analysis also proved to be a fast and accurate semiquantitative test, providing a useful estimate of tissue ivermectin concentrations. Furthermore, it is evident from Figs 3 and 5 that the cases C998-06 and C999-06 provided convincing matches to the mass spectral peaks of the ivermectin standard, unequivocally confirming the administration of this agent to these animals.

There have been increasing concerns in recent years about the safety of the anthelmintic ivermectin (Fig. 2). This concern occurs in spite of its wide applicability ranging from roundworms (cattle, swine, dogs, cats) to grubs (bison) and warbles (reindeer), its recognized safety in human medicine and even its potential as an insecticide for Eastern tent caterpillars.

Concerns arise particularly because of its expansion in use and availability. This case study shows how commonly used medications, like ivermectin, can become fatal if not administered properly. Anthelmintics can be administered in a number of different ways including oral, intravascular, subcutaneous and as a pour-on, with drench, paste, injectable and pour-on formulations available. These varying methods enable suitability to various clinical situations, with specificity for different animal types. It is important that the manufacturer’s instructions be adhered to with regard to the spectrum of activity, the animal subtypes for which it is effective, the dosage.

As presented here, this ESI-MS/MS method was intended as a rapid diagnostic test providing unequivocal identification of the presence of ivermectins and semiquantitative estimates of tissue concentrations. Pulliam and Preston stated that a subcutaneous presence of ivermectins and semiquantitative estimates of tissue concentrations in collies sensitive to ivermectin-induced toxicosis. Furthermore, it is evident from Figs 3 and 5 that the cases C998-06 and C999-06 provided convincing matches to the mass spectral peaks of the ivermectin standard, unequivocally confirming the administration of this agent to these animals.

Risks in certain patients include itching, swollen lymph glands, hypotension, fever, dizziness, headache and myalgia. These side effects are reported to occur most frequently in patients suffering from high microfilaria counts. Veterinarians and farm workers who are involved in treating animals for ectoparasites with ivermectin are also at risk. According to Temple and Smith,

As with canines, ivermectin has also found use in human medicine. For humans, ivermectin is widely used for the treatment and death in two different species of lightweight canines. Our case study revealed that ivermectin at ~30–50 mg/kg induced dilated pupils, ataxia, cardiac arrest and death in two different species of lightweight canines.

In closing, this ethanol extraction/filtration approach to ivermectin overdose cases may have considerable application in diagnostic work. The method clearly and unambiguously identified ivermectin, at concentrations consistent with chemical overdose in two canines. The method should be easily adapted to a fully validated quantitative method which would have applicability to human and environmental issues when these compounds are used on livestock or other food sources.

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References


