ORIGINAL ARTICLE

Quality of amoxicillin/clavulanic acid oral formulations for intended veterinary use in the UK, Malaysia, Serbia and Thailand

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Objectives: Amoxicillin/clavulanate is the most commonly used oral antimicrobial drug in companion animals. The objective of the study was to detect types and frequency of deficits in the quality of veterinary oral formulations of amoxicillin/clavulanate in various countries.

Materials and Methods: In a prospective study with purposive sampling, amoxicillin/clavulanate tablet formulations for canine use were collected in four countries (wholesalers or veterinary practice) and shipped to a central bioanalytical laboratory. Twenty-four samples were collected from the UK (nine), Malaysia (nine), Serbia (four) and Thailand (two), yielding 18 different formulations (10 veterinary). Packaging inspection, tablet disintegration and content assay were conducted (validated high-performance liquid chromatography with ultra-violet detection); content was acceptable when within the 90% to 120% pre-specified range (US Pharmacopeia).

Results: Secondary packaging was present for 13 of 24 samples and primary packaging integrity was verified for all but one sample. Amoxicillin trihydrate/potassium clavulanate label ratio was 4:1, except for three formulations (2:1). Tablet dose strength ranged from 250 to 625 mg. All formulations contained both analytes. For amoxicillin, two of 24 samples were out of specification with 72.8% (Malaysia) and 82.3% (Thailand) of labelled content. For clavulanate, four of 24 samples were out of specification with 46.9% (Serbia), 79.0% (UK), 84.3% (Serbia) and 86.5% (Thailand) of labelled content. One formulation (Thailand) failed for both analytes.

[a]Members of the WSAVA TGG are Stephen Page, Guillermina Manigot, Melissa Bourgeois, Devon Dublin, Scott Weese, Ludovic Pelligand, Elzo Kannekens, Andreja Kastelic Hrcek, Felisbina Queiroga, Walt Ingwersen, Paulo Steagall (co-chair), Luca Guardabassi (Chair), Information was disseminated with presentation at the World Small Animal Veterinary Association congress (Lima Oct 2022).

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Clinical Significance: Antimicrobial formulations of substandard quality have negative consequences for efficacy in patients and potentially promote antimicrobial resistance. There was evidence of substandard formulations in all countries, not only for amoxicillin but especially for clavulanate; this could compromise equitable access to acceptable quality essential veterinary medicines worldwide.

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INTRODUCTION

In 2020, the WSAVA (World Small Animal Veterinary Association) Therapeutic Guidelines Group (TGG) published the Essential List of Medicines in cats and dogs (Steagall *et al.* 2020, WSAVA TGG 2020), mirroring the WHO Essential Medicines List (World Health Organization 2021). The list details core and complementary medicines veterinarians around the world should have access to for fulfilling basic veterinary care and ensuring animal welfare of companion animals. A subsequent question concerns drug quality in countries where these medicines could be purchased. Poor quality medicines were defined by the 17th World Health Assembly (World Health Organization 2022) as (1) falsified (F) formulations which deliberately/fraudulently misrepresent their identity, composition or source or (2) substandard (S) formulations which are authorised medical products that fail to meet either their quality standards or their specification, as defined in the US Pharmacopeia (USP). Substandard or falsified (SF) formulations have deleterious health, economic and reputational impact. Lack of efficacy due to SF veterinary products may be life threatening in the case of antimicrobial drugs or vaccines. Furthermore, SF antimicrobial products are likely to expose sick animals to sub-therapeutic levels and contribute to treatment failure and antimicrobial resistance (Zabala *et al.* 2022).

According to the WHO, the percentage of observed failure rate of analysed medical product samples from low- and middle-income countries was 10.5% [95% confidence interval (CI) 9.9 to 11.0] and 10.6% (10.3 to 10.9%), respectively (World Health Organization 2020). When focussing on antibiotics only, the percentage of observed failure rate was 7.2% (95% CI 6.8 to 7.7). The prevalence studies for SF veterinary antibiotic medicines are rare (13 studies in the last 20 years) (Vidhamaly *et al.* 2022). These studies mainly focus on production animal formulations in Asia and Africa; they report failure rates between 11% and 93%, depending on the study design (Pellicaan *et al.* 1996, Gberindyer *et al.* 2019, Vidhamaly *et al.* 2022). In Europe, a small amount of the production is monitored by the manufacturer and by the official medicines control laboratories (Official Medicines Control Laboratories 2022), under the direction of the European Directorate for the Quality of Medicines, but SF formulations may still be encountered.

Oral amoxicillin and clavulanic acid (AMC) fixed dose combination is one of the most commonly used antimicrobial treatments in companion animals worldwide. Depending on local regulations, product licences for both veterinary and human use are encountered in the field of small animals therapeutics. Both active ingredients are sensitive to degradation and it is therefore important to monitor the content of both actives, to ensure adequate exposure of treated animals. The objective of the study was to detect types and frequency of deficits in the quality of oral formulations of AMC purchased for small animal use in four countries (Malaysia, Serbia, Thailand and UK).

MATERIALS AND METHODS

A feasibility survey was sent to representatives of WSAVA in June 2020 (ethical approval URN SR2020-0217). Replies from 28 countries were received (three Africa, 13 Europe, six America, four Asia and two Oceania). All but one country had amoxicillin clavulanic tablets available for dogs, 22 countries declared willingness to participate voluntarily in this study on medicine quality and 16 were aware of possible restrictions to shipping medicines abroad. The representatives of each non-EU countries were asked to provide formulations and we received samples from four countries (Malaysia, Serbia, Thailand and UK) between January and October 2021. Samples were analysed between December 2021 and July 2022. Sampled outlets were veterinary practices, university hospital pharmacies and wholesalers. For reporting study results, the Medicine Quality Assessment Reporting Guidelines (MEDQUARG) (Newton *et al.* 2009) and the aforementioned SF product definitions were considered. MEDQUARG is a reporting standard for medicine quality surveys that includes a list of 26 desirable items.

Sampling design and sample size calculation

The minimal number of outlets to sample was determined using the random Lot Quality Assurance Sampling (LQAS) approach, as recommended in the MEDQUARG guideline (Newton *et al.* 2009). It pragmatically uses small sample sizes to classify results as "acceptable" *versus* "unacceptable" (Lemeshow & Taber 1991). Assuming an overall prevalence of 7.2% of SF antimicrobials and that there is no difference between the veterinary and human supply chain, we set up the acceptable proportion (100%, zero tolerance) and the unacceptable (92.8%, average prevalence) proportion of compliant medicines, with maximal alpha and beta errors of 5% and 10%, respectively. The detection of one outlet selling SF in a sample size of 31 would correspond to an unacceptable result (Newton *et al.* 2009). This figure informed our sample size calculation.

Any amoxicillin clavulanic acid tablet formulations that could be used locally to treat companion animals (veterinary or

human products) were eligible. A minimum of 32 tablets per formulation were required for analysis. When possible, two geographic areas were sampled within a country. It was possible to ship the same formulation but from different outlets. Any tablet with at least 250 mg of the combination could be collected. AMC tablets were required to be stored and protected from light between 15 and 25°C at 30% to 45% humidity before and after shipment. Prospective purposive non-randomised sampling was applied. Any country member of the WSAVA was eligible for sampling.

Samples were collected by samplers and shipped to the country coordinator who organised shipment with a courier company (tracked delivery) to the central laboratory located in the UK (Fig 1). The choice of a single central analytical laboratory, after a tender process (best bid out of four), removed the effect of between-laboratory variability. Samplers and providers of formulations were not blinded and received full disclosure of the aim of the study. Compensation was provided for purchase of the medicine and shipping cost.

Ethical approval

The study received approval from the Clinical Research Ethical Review Board (URN 2020 1987-2) of the Royal Veterinary College. As the scientific purpose was fully disclosed, the study used neither false prescriptions nor mystery shopper approaches. Importation into the UK was facilitated by official confirmation that no further approval or certification was required, under the Veterinary Medicines Regulations 2013, to undertake the importation of these products for the purpose of undertaking quantitative analysis. The quantities sampled were too low to be detrimental to the supply chain of the country of origin.

Packaging inspection and quality analysis

Visual inspection of the physical characteristics of dosage form, packaging and labelling information was performed. All samples underwent visual inspection of trade name, active ingredient name, the manufacturer's name and full address, labelled medicine strength, dosage form, number of units per container, batch/lot number, manufacturing and expiry dates, presence of leaflets/package insert and whether the formulation was locally manufactured or imported. Visual inspection was performed encompassing uniformity of colour and size, breaks, cracks, splits, embedded surface spots or visually obvious contamination. Uniformity of weight, disintegration in simulated gastric fluid and content assay tests according to the USP were carried out (United States Pharmacopeia 2009). Data on assay content and weight were reported as mean±sd. Method accuracy metrics are detailed in the method validation section.

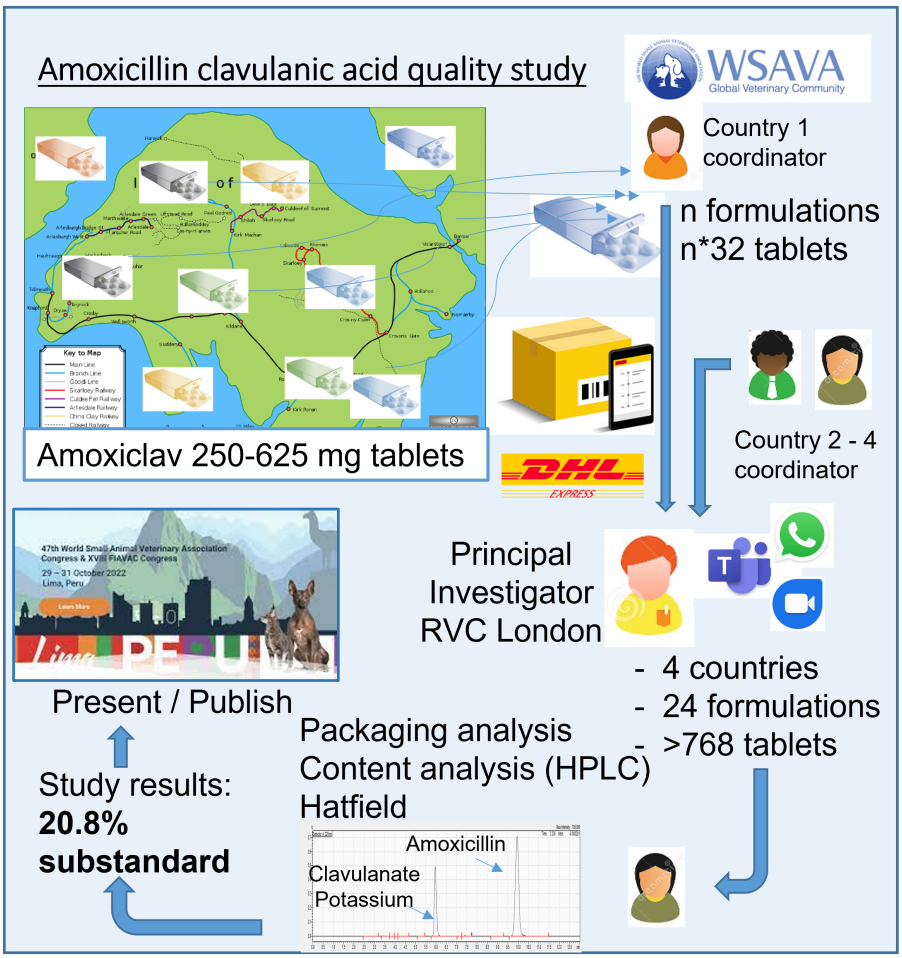

FIG 1. Workflow for study on quality of amoxicillin clavulanic acid tablets for veterinary use

Method validation and chemical analysis

Dose content analysis was performed on the formulations using assay methodology derived from the USP monograph for Amoxicillin and Clavulanate Potassium Tablets (United States Pharmacopeia 2009). The analysis was not performed blinded to packaging. Analytical grade standards were purchased (Sigma, Poole, Dorset) to make standard solutions of 0.5 mg/mL amoxicillin trihydrate and 0.2 mg/mL of clavulanate potassium in high-performance liquid chromatography (HPLC) grade water.

For the analysis of the samples, 10 tablets were weighed out before being crushed with a pestle and mortar for 5 minutes. One dose of formulation (based upon an average tablet weight derived from the 10 tablets) was weighed out in triplicate into 500 mL volumetric flasks to which deionised water was then added (Fig 2). This was then stirred for 60 minutes at 500 rpm using a magnetic stirrer. A 50 mL aliquot of the samples was filtered (0.2 µm PTFE, Fisher Scientific, Loughborough, UK) and the samples were diluted, using water as a diluent, to bring the concentration of amoxicillin and clavulanate into the validated assay range. Practically, most samples were diluted in two different ways: (1) 3 parts in 5, by taking 15 mL of the sample and making up to 25 mL with diluent (water), and (2) 4 parts in 5, by taking 20 mL and making up to 25 mL with diluent. This step brings the amoxicillin and clavulanate concentration in the sample close to the target concentration of the assay. The samples were mixed for 10 seconds using a vortex mixer before dispensation into HPLC vials and injection of 10 µL into the HPLC system.

The HPLC mobile phase was made of 1:19 methanol and buffer (7.8 g of monobasic sodium phosphate in 900 mL of water, adjusted to a pH of 4.4±0.1 and diluted with water to 1000 mL). Chromatographic separation was performed using the mobile phase, as described above, flowing at a rate of 2 mL/minute over an ODS Hypersil column containing packing material of L1 type (4 mm×30 cm, 5 µm). The detector wavelength was set at 220 nm, as per USP methodology. An analytical standard solution was injected for five replicates before every batch of, acting as a system suitability test (SST), to confirm that the instrument and analytical method were performing as expected, and in line with noted parameters in the USP monograph [relative standard deviation (RSD) <2%, tailing factor <1.5, resolution between analytes >3.5] (Fig 3). Acceptability of recovery of the actives from each formulation was evaluated, based upon USP criteria (90% to 120% for both amoxicillin and clavulanate potassium) (United States Pharmacopeia 2009).

For validation, instrument linearity was assessed by analysis of standard solutions at a range of 50% to 150% around the target concentrations of interest. These ranges were 0.25 to 0.75 mg/mL for amoxicillin and 0.1 to 0.3 mg/mL for clavulanate potassium. Acceptable linearity was observed (R^2>0.999) (Fig S1). The precision and the accuracy of the method were assessed by repeat analysis of solutions of analyte at 70%, 100% and 130% of the target concentrations. These solutions were prepared by weighing an appropriate amount of reference standard, diluting with 20 mL of water, sonicating for 10 minutes, and stirring for 30 minutes at 300 rpm. For amoxicillin, this covered concentrations of 0.35, 0.5 and 0.65 mg/mL. For clavulanate potassium, this covered concentrations of 0.14, 0.2 and 0.26 mg/mL. The concentration of these solutions was then calculated by use of a single-point calibration around the target concentration of 0.5 and 0.2 mg/mL for amoxicillin and clavulanate potassium, respectively. This calculation is given in equation 1 and was used for all accuracy standards and formulation samples.

$$\frac{\text{Standard or Sample Response}}{\text{Response Factor}} = \text{Concentration}\ (\text{mg/mL}) \quad (1)$$

Accuracy was assessed by measuring the closeness of calculated concentrations of standards, at the above-mentioned concentrations of 70%, 100% and 130% of target concentration, to the theoretical concentration based upon the weighed amount of API. Three repeats of standards were prepared at each concentration. Equation 2 expresses this calculation:

$$\%\text{Accuracy} = \frac{\text{Measured Concentration (by HPLC determination)}}{\text{Theoretical Concentration (by weighing)}} \times 100 \quad (2)$$

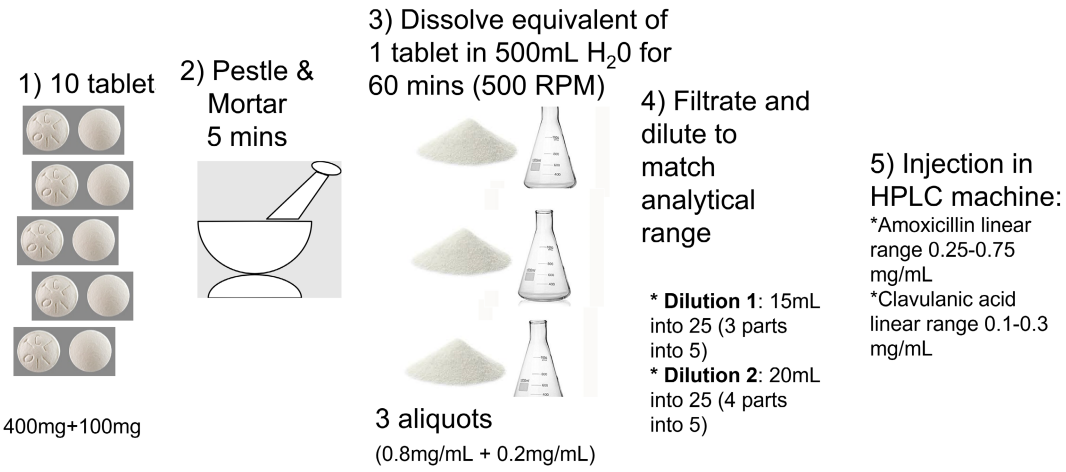

FIG 2. Sample processing procedure for amoxicillin clavulanic acid tablets before injection into the column of the HPLC machine

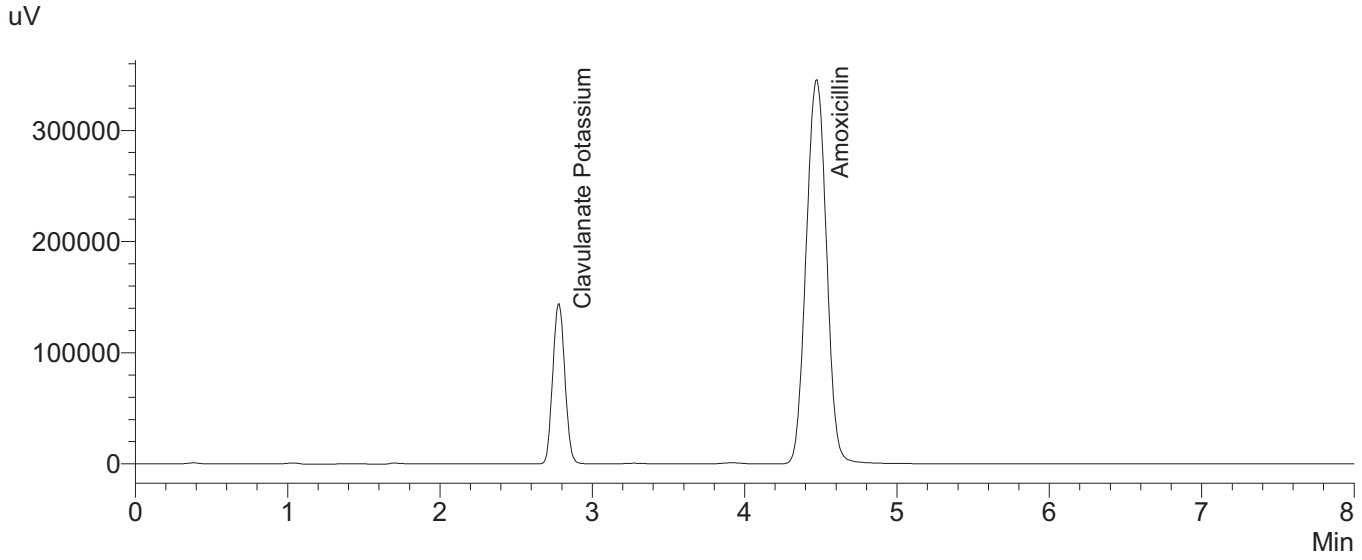

FIG 3. Representative chromatograms at Target Concentration (0.5 mg/mL for Amoxicillin, 0.2 mg/mL for Clavulanate Potassium). Clavulanate is eluting first at 2.78, with amoxicillin eluting second at 4.47 minutes

These accuracy standards were then injected six times to assess the precision of the method. Repeated measurements were used to calculate %RSD, as a value of method precision. Acceptable accuracy (<2%) and precision (<2%) were observed for the tested concentrations (Table S1).

RESULTS

Outlets sampled and missing samples

Twenty-four samples were collected from Malaysia (9), the UK (9), Serbia (4) and Thailand (2). As 6 samples were repeat samples from the same brand and manufacturer but from another geographical location, the study hence included 18 different formulations (10 veterinary ones). All the samples from the UK and Thailand were ordered through the wholesaler supplying the pharmacy service of one veterinary teaching hospital. Samples from other countries were collected from private practices or pharmacies of veterinary hospitals. None of the samples were collected from unregulated markets (*e.g.* online without prescription). Some samples were collected but could not reach the central laboratory: two samples could not be sent from Serbia, due to issues at border inspection. Also, samples were collected in another country of central America but could not be shipped because of an unsurmountable administrative barrier to exportation.

Packaging and quality analysis results

Secondary packaging was present for 14 of the 24 samples. Half of the formulations were initially destined for human use (eight for Malaysia, two for Serbia, one for the UK and Thailand), the rest were veterinary formulations. The ratio of amoxicillin trihydrate/potassium clavulanate was 4:1, except for three human formulations for which it was 2:1. Tablet dose strengths were 250 mg (three formulations), 375 mg (three formulations, all with a 2:1 ratio), 500 mg (eight formulations) and 625 mg (10 formulations). Median number of tablets per sample was 32. Primary packaging integrity was verified except in one case (broken blister for one of the six tablets, all other five blisters intact), this particular blister was not analysed.

For uniformity of weight, the average tablet weights were 623±246 mg, 673±1 mg, 1409±291 mg and 1078±74 mg, for the 250, 375, 500 and 625 mg formulations, respectively. The average coefficient of variation on 10 tablets ranged between 0.46% and 2.28% (only two formulations above 2%). When formulations were tested for disintegration, one out of 24 failed to disintegrate in simulated gastric fluid within 30 minutes (United States Pharmacopeia 2009).

For assay content, all formulations contained both of the drugs, but content varied. For amoxicillin, 22 out of 24 formulations met the target assay content range (90% to 120%), with an average content of 97.63%±2.77% for compliant formulations (Table 1). For clavulanic acid, 20 out of 24 formulations had a content within the pre-specified range (90% to 120%), with an average content of 103.65%±1.79% for compliant formulations (Table 2). One formulation failed for both analytes.

Category of poor-quality medicine

None of the failing formulations were past their expiry date. For amoxicillin, two samples were out of specifications and designated as substandard. One veterinary formulation from Malaysia (250 mg, sampled July 15, 2021, analysed July 19, 2022, expiry date March 30, 2023) contained 72.8% and 106.25% of AMC label content, respectively. This formulation (number 8) showed a RSD percentage higher than 10% (Table 1). One veterinary formulation from Thailand (250 mg, collected October 11, 2021, analysed July 21, 2022, expiry date March 17, 2023) failed for both analytes, with contents of 82.35% and 86.49% of label claim for AMC, respectively. For clavulanic acid, another three samples were out of specification and also

Table 1. Formulations tested and amoxicillin content assay results out of three replicate measurements obtained from a pool of 10 tablets

Origin	Amoxicillin (assay content acceptable limits 90% to 120%)									
	Formulation number	Sampling date	Expiry date	Assay date	Repeat (%)			Statistics (%)		
					1	2	3	Average	sd	RSD
UK	0	January 19, 2021	May 1, 2022	December 17, 2021	94.47	99.09	98.27	97.3	2.5	2.5
UK	1	March 2, 2021	June 30, 2024	February 11, 2022	100.27	100.83	93.87	98.3	3.9	3.9
UK	2	March 2, 2021	October 1, 2021	March 3, 2022	103.90	100.42	104.52	102.9	2.2	2.1
UK	3	March 2, 2021	January 29, 2023	July 19, 2022	100.70	88.29	83.95	91.0	8.7	9.6
UK	4	March 2, 2021	October 1, 2023	March 3, 2022	95.52	105.25	98.65	99.8	5.0	5.0
UK	5	March 2, 2021	June 1, 2023	May 20, 2022	96.18	100.36	97.32	98.0	2.2	2.2
Malaysia	6	April 21, 2021	June 1, 2022	May 27, 2022	99.54	99.36	94.02	97.6	3.1	3.2
Malaysia	7	April 21, 2021	July 1, 2021	May 26, 2022	101.96	101.12	100.91	101.3	0.6	0.6
Malaysia	8	July 15, 2021	March 30, 2023	July 19, 2022	84.68	63.65	70.11	72.8	10.8	14.8
Malaysia	9	April 21, 2021	May 1, 2022	May 20, 2022	102.04	101.37	100.37	101.3	0.8	0.8
Malaysia	10	April 21, 2021	August 1, 2022	April 13, 2022	97.49	95.87	97.45	96.9	0.9	1.0
Malaysia	11	April 21, 2021	June 1, 2022	April 14, 2022	99.35	94.75	98.49	97.5	2.4	2.5
Malaysia	12	April 21, 2021	April 1, 2023	May 26, 2022	104.07	104.90	103.28	104.1	0.8	0.8
Malaysia	13	April 21, 2021	August 1, 2022	May 27, 2022	97.13	89.92	89.98	92.3	4.1	4.5
Malaysia	14	April 21, 2021	June 1, 2022	May 27, 2022	97.36	96.25	83.72	92.4	7.6	8.2
Thailand	15	October 11, 2021	March 17, 2023	July 21, 2022	83.62	81.63	81.80	82.4	1.1	1.3
Thailand	16	October 11, 2021	April 22, 2023	May 26, 2022	105.60	105.81	99.96	103.8	3.3	3.2
Serbia	17	May 11, 2021	February 1, 2024	July 20, 2022	92.38	91.04	92.10	91.8	0.7	0.8
Serbia	18	May 11, 2021	October 1, 2023	July 20, 2022	98.75	94.95	90.46	94.7	4.1	4.4
Serbia	19	May 11, 2021	August 1, 2022	July 21, 2022	100.09	100.36	99.39	99.9	0.5	0.5
Serbia	20	May 11, 2021	December 1, 2021	July 21, 2022	99.15	99.62	98.53	99.1	0.5	0.6
UK	21	October 13, 2022	December 1, 2023	December 1, 2022	97.92	100.09	98.67	98.9	1.1	1.1
UK	22	October 13, 2022	May 3, 2024	December 1, 2022	93.23	93.86	84.10	90.4	5.5	6.0
UK	23	October 13, 2022	August 1, 2024	November 30, 2022	98.52	97.89	98.40	98.3	0.33	0.34

sd Standard deviation, RSD Relative standard deviation (%)
Orange highlight: failed replicate. Green highlight: formulation satisfies US Pharmacopeia specifications (90% to 120%). Red highlight: failed formulation

Table 2. Formulations tested and clavulanic acid content assay results out of three replicate measurements obtained from a pool of 10 tablets

Origin	Clavulanate (assay content acceptable limits 90% to 120%)									
	Formulation number	Sampling date	Expiry date	Assay date	Repeat (%)			Statistics (%)		
					1	2	3	Average	sd	RSD
UK	0	January 19, 2021	May 1, 2022	December 17, 2021	112.66	109.37	108.58	110.2	2.2	2.0
UK	1	March 2, 2021	June 30, 2024	February 11, 2022	98.93	100.20	97.84	99.0	1.2	1.2
UK	2	March 2, 2021	October 1, 2021	March 3, 2022	102.08	101.92	102.79	102.3	0.5	0.5
UK	3	March 2, 2021	January 29, 2023	July 19, 2022	78.21	81.02	77.68	79.0	1.8	2.3
UK	4	March 2, 2021	October 1, 2023	March 3, 2022	105.09	104.27	98.68	102.7	3.5	3.4
UK	5	March 2, 2021	June 1, 2023	May 20, 2022	105.35	100.26	100.65	102.1	2.8	2.8
Malaysia	6	April 21, 2021	June 1, 2022	May 27, 2022	95.02	94.73	95.40	95.0	0.3	0.4
Malaysia	7	April 21, 2021	July 1, 2021	May 26, 2022	106.41	106.15	108.12	106.9	1.1	1.0
Malaysia	8	July 15, 2021	March 30, 2023	July 19, 2022	103.01	109.75	106.00	106.3	3.4	3.2
Malaysia	9	April 21, 2021	May 1, 2022	May 20, 2022	100.41	100.16	102.28	101.0	1.2	1.1
Malaysia	10	April 21, 2021	August 1, 2022	April 13, 2022	100.35	99.88	98.70	99.6	0.8	0.8
Malaysia	11	April 21, 2021	June 1, 2022	April 14, 2022	96.61	95.57	82.98	91.7	7.6	8.3
Malaysia	12	April 21, 2021	April 1, 2023	May 26, 2022	112.64	112.51	112.23	112.5	0.2	0.2
Malaysia	13	April 21, 2021	August 1, 2022	May 27, 2022	98.64	100.97	99.57	99.7	1.2	1.2
Malaysia	14	April 21, 2021	June 1, 2022	May 27, 2022	100.74	101.72	100.49	101.0	0.7	0.6
Thailand	15	October 11, 2021	March 17, 2023	July 21, 2022	85.43	86.36	87.67	86.5	1.1	1.3
Thailand	16	October 11, 2021	April 22, 2023	May 26, 2022	108.84	111.79	109.97	110.2	1.5	1.4
Serbia	17	October 20, 2021	February 1, 2024	July 20, 2022	84.86	84.63	83.40	84.3	0.8	0.9
Serbia	18	October 20, 2021	October 1, 2023	July 20, 2022	45.70	46.74	48.21	46.9	1.3	2.7
Serbia	19	October 20, 2021	August 1, 2022	July 21, 2022	105.46	104.24	104.92	104.9	0.6	0.6
Serbia	20	October 20, 2021	December 1, 2021	July 21, 2022	106.38	105.63	105.26	105.8	0.6	0.5
UK	21	October 13, 2022	December 1, 2023	December 1, 2022	111.87	107.21	109.79	109.6	2.3	2.1
UK	22	October 13, 2022	May 3, 2024	December 1, 2022	110.56	109.57	108.92	109.7	0.8	0.8
UK	23	October 13, 2022	August 1, 2024	November 30, 2022	102.01	106.72	100.07	102.9	3.4	3.3

sd Standard deviation, RSD Relative standard deviation (%)
Orange highlight: failed replicate. Green highlight: formulation satisfies US Pharmacopeia specifications (90% to 120%). Red highlight: failed formulation

recognised as substandard. One veterinary formulation from Serbia (500 mg, sampled October 20, 2021, analysed July 20, 2022, expiry date October 1, 2023) contained only 46.9% of clavulanic acid but 94.7% of amoxicillin label content. The same formulation with a different batch number (500 mg, expiry date February 1, 2024) sampled in a different part of the country, had 84.3% clavulanic acid label content but acceptable amoxicillin content (91.8%). One veterinary formulation from the UK had a low clavulanic acid content of 79.0% but an acceptable amoxicillin content (91.0% of the label content).

No packaging features allowed the identification of substandard formulations. The name of the companies was kept anonymous, all companies were informed when assay results were out of specifications but no reporting was made to regulatory authorities.

DISCUSSION

A recent systematic literature search of previous studies reporting quality of veterinary medicines (Vidhamaly *et al.* 2022) screened PubMed, Embase, MEDLINE, Global Health, Web of Science, CAB Abstracts, Scopus and Google searches ranked by relevance order. It identified 20 studies with commercial formulations used for exclusively treatment of livestock and fish in single countries that were not classified as high-income countries. Our study is the first one to evaluate the quality of an oral antibiotic combination for small animal use globally, with the inclusion of sampling within higher- and lesser-income countries. In the European Union, samples of formulations are tested by drug companies before batch release; additionally, post-marketing assessment of pharmaceutical products (human and veterinary) is carried out episodically by the European Medicines Agency and European Directorate for the Quality of Medicines & HealthCare for centrally authorised medicinal products. Over 20 years, the European Medicines Agency identified that 9% of the tested veterinary medicines had technical or regulatory issues, only a fraction of this was due to products out of specifications (European Medicines Agency 2019). Our study constitutes a pilot investigation that identified issues with drug quality and helps draw a roadmap to more global surveillance for quantitative evaluation of the quality of veterinary drugs.

We identified several formulations as substandard for either or both of the actives and no country was exempt from formulations falling out of specification. Global prevalence rate of failure from at least one of the active was 20.8%, but due to the small sample size, the CI of this proportion is wide [binomial proportion 95% CI: (7.1% to 42.2%), Clopper-Pearson exact method]. However, it was not possible to distinguish between substandard quality as a result of (1) non-compliance with good manufacturing practice *versus* (2) post-production degradation due to inappropriate storage (Vidhamaly *et al.* 2022). It is possible that some of the formulations that included degraded products left the factory as formulations of good quality. Degradation is possible with both AMC. While these could not be excluded, we strived to limit exposure to uncontrolled conditions with the use of fast and tracked couriers. Besides, there was no association between failure incidence and containment in a specific parcel. The higher

failure incidence observed for clavulanic acid might be explained by the lower stability of this β-lactamase inhibitor compared to amoxicillin (Khan *et al.* 2013).

Assay of each formulation was performed by the crushing of 10 tablets, from which the equivalent of three doses of formulation was weighed out and API content assessed. Whilst the values for RSD observed across these three replicates were generally acceptable for the formulations tested, several formulations had higher than usual extraction variability, particularly when consideration was given to amoxicillin content. Though these values are not unusual in comparison to existing literature, they could be indicative of poor content uniformity or reduced assay precision (Seifu *et al.* 2019). The aqueous solubility of amoxicillin trihydrate is typically reported in the range of 1 to 10 mg/mL (Thambavita *et al.* 2017). Given the proximity of the assay concentration (0.5 mg/mL) to this range, it may be the case that amoxicillin solubility influences the variability of the extraction, with higher RSDs resulting. Poor content uniformity of the tested formulations may also be implicated; however, it is challenging to assess this without performing assay of the formulations a greater number of times. The low number of collected tablets prohibited greater investigation into this aspect of the study.

In the present study, that included both human and veterinary formulations, we did not find evidence of falsified medicines, where the actives could not be retrieved. Falsified formulations are a problem reported for AMC, as in the last 4 years only, there have been three reports of Falsified Augmentin formulations (World Health Organization 2018, 2019a,b).

The deviations from target content up to 10% are unlikely to affect the therapeutic exposure significantly but deviations from 20% to 50%, like we observed in several products, could substantially reduce the time for which free plasma concentration exceeds the minimal inhibitory concentration (MIC) of pathogens. As beta-lactams, like amoxicillin, are time-dependent antimicrobials, this could lead to decreased efficacy and possible clinical failure. Selection of antimicrobial resistance (AMR) by bacterial strains can occur due to low bioavailability of amoxicillin or insufficient protection against beta-lactamases with low availability of clavulanic acid. AMR selection, in turn, poses risks to both animal and public health due to the close link between owners and their pets (Vidhamaly *et al.* 2022, Zabala *et al.* 2022). Subtherapeutic exposure due to substandard formulations can contribute to the selection of extended-spectrum beta-lactamase (ESBL) producing bacteria, which increase mortality and cost of care globally due to limited therapeutic options.

Appropriate intervention on our part was to present a summary of this work at the 2022 WSAVA congress and to publish the full report in the public domain. We notified drug manufacturers of failed formulations and offered the remaining tablets for retest at their facilities.

There were several limitations to our study. Firstly, this study was not randomised; a more appropriate sampling design for this study would be stratified random sampling. Although this was an initial aspiration of ours, the availability of the sampling team or individual in each country did not allow the mapping of many sites and random selection of sampling location, as we had limited resources and this study took place during the peak of the COVID-19 pandemic.

The second limitation is the small sampling size overall and in each country. Despite the small sampling size and the facilitation to enter the country of analysis, the precision of the prevalence estimate is low and must be refined with more extensive sampling within countries. The choice of the countries depended on the motivation of the individuals and the willingness to represent countries in Europe, could also have led to introducing bias. Although sufficient funding was available, the limitations to sample size were attributed to the study logistics, with chiefly two reasons. First, local bureaucracies complicated the shipping samples, especially in South and Central America (some countries could not submit their samples or would not start collection due to anticipated barriers). We have to report the case of one formulation we could not test, as, when inspected at border control, local drug company opposed the shipment of the samples. The second reason was an overall difficulty to follow-up the sampling actions in a number of countries after initial engagement and agreement to participate in the feasibility study. These difficulties persisted despite the multiplicity of media to maintain engagement of the local teams (email, What's app, phone calls). Lack of local investigator's time due to changing priorities within the COVID pandemic is a possible explanation. In an ideal setting, subsequent medicine quality studies should include (1) randomised sampling of medicine, (2) provision to legally enable a 'mystery shopper' sampling approach to mimic real-life procurement, (3) data logger-documentation of storage conditions and minimisation of the time between collection and analysis and (4) support by a motivated local team and governments with representation of different OIE regions (Africa, Asia, Americas and Europe) (Zabala *et al.* 2022).

In conclusion, there was evidence of substandard formulations in each country, especially for clavulanic acid, but also for amoxicillin. This finding supports the efforts of the WSAVA TGG to promote equitable access to essential veterinary medicines to practitioners to all countries. Substandard antibiotics are likely to impede achieving the Sustainable Development Goals and to promote AMR in pathogens shared between humans and animals.

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Author contributions

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porting); writing – review and editing (supporting). **Jacqueline L Stair:** Conceptualization (supporting); methodology (equal); resources (supporting); software (supporting); validation (supporting); writing – review and editing (supporting). **Mark Scott:** Conceptualization (supporting); methodology (equal); software (supporting); validation (supporting); writing – review and editing (supporting). **Fang Liu:** Conceptualization (supporting); methodology (equal); software (supporting); validation (supporting); writing – review and editing (supporting). **Stephen W Page:** Conceptualization (supporting); methodology (supporting); supervision (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Luca Guardabassi:** Conceptualization (equal); funding acquisition (equal); resources (equal); writing – review and editing (equal). **Paulo V. Steagall:** Conceptualization (equal); funding acquisition (equal); resources (equal); writing – review and editing (equal).

Conflict of interest

Zoetis is a sponsor of the WSAVA TGG, but did not have any input in design, execution or write up of the study. None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. linearity of the instrument response around the target concentrations of interest, 0.5 mg/mL of Amoxicillin Trihydrate and 0.2 mg/mL of Clavulanate Potassium.

Table S1. Results for accuracy and precision of the standards.