Fosfomycin concentrations in epithelial lining fluid in weaning piglets

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Respiratory diseases are one of the most important problems in modern intensive swine production. These diseases are a common cause of morbidity and mortality in weaning pigs. The concentrations of antibiotics in epithelial lining fluid (ELF) reflect the antimicrobial activity for extracellular pathogens involved in respiratory diseases (Schentag & Ballow, 1991; Kiem & Schentag, 2008). Fosfomycin is a hydrosoluble bactericidal broad-spectrum antibiotic used in Central and South America and various Asian countries. Although fosfomycin showed clinical efficacy in the treatment of pulmonary diseases, concentrations in ELF have not still been established in any species. Assuming that the determination of fosfomycin concentration in ELF (biophase) represents the key parameter for establishing efficacy of antibiotics, the objective of this work was to characterize the potential penetration of fosfomycin in ELF and its relationship with serum concentrations in weaning piglets.

Six weaning piglets (three males and three females), clinically healthy 25–28 days old, were used in this trial. To minimize the stress and facilitate blood sampling, a permanent long catheter was placed in each piglet in the left external jugular vein according to the method of Soraci *et al.* (2010). Serum concentrations of disodium-fosfomycin were evaluated following a single i.m. dose of 15 mg/kg in the gluteus muscle. The disodium-fosfomycin was supplied by Bedson S.A. Laboratories, Pilar, Buenos Aires, Argentina. It was dissolved in a 10% sodium citrate solution that yielded a pH of 6.8. The study was carried out following the rules of ethical approval by the experimental ethics committee of Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Argentina. To obtain the bronchoalveolar lavage fluid (BALF), a flexible fiber optic bronchoscope (Olympus BF-P20D) was used. The bronchoscope was pushed into the bronchus trachealis which leads into the right cranial lung lobe (Shields & Riedler, 2000; Baltes *et al.*, 2001; Scollo *et al.*, 2011). Seven milliliters of sterile 0.9% saline (prewarmed to 30 °C) was introduced and recovered by using a vacuum pump aspiration with a maximum of 15 kPa (Shields & Riedler, 2000; Baltes *et al.*, 2001). This washing was repeated three times and a range between 15 and 18 mL was obtained (Shields & Riedler, 2000). The procedure of instillation and collection was completed in <1 min (the average dwell time was about 50 ± 10 sec) (Baughman *et al.*, 1983; Dohn & Baughman, 1985; Rennard *et al.*, 1986; Grigg *et al.*, 1991; Lamer *et al.*, 1993; Baughman, 1997; Mombarg *et al.*, 2002). The aliquots were pooled for analysis. Blood and BALF samples were collected at the same time after fosfomycin i.m. administration: 1, 2, 4, 6, 8, and 12 h. The serum was separated immediately by centrifugation at 2000 g for 15 min and frozen at −20 °C until analysis. The lavage sample was centrifuged immediately at 400 g for 10 min, and the supernatant was separated from the pellets. Fosfomycin concentrations in serum and dilute solution of BALF were measured using a high-performance liquid chromatography–mass-mass spectrometry (HPLC-MS/MS) according to the method determined by Scollo *et al.* (2011). Estimation of the amount of ELF sampled by BALF was performed using the urea dilution method (Taylor *et al.*, 1956; Theodore *et al.*, 1975; Rennard *et al.*, 1986). The urea content was measured in BALF and serum according to the urea test kit instructions (Urea testkit; Sigma Chemical, St Louis, MO, USA).

The AUCs of fosfomycin in ELF and serum were calculated by the trapezoidal rule when multiple measurements were available. Paired *t*-test was used to compare pharmacokinetics data by using a SAS software package (SAS Institute Inc., Cary, NC, USA). A P value of <0.05 was regarded as statistically significant. The samples of BALF collected were clear and free of blood, mucus, and debris. In contrast to human, dog, cat, and

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horse, the lungs of cattle, pigs, and sheep are markedly segmented, so that the (BALF) sample is only considered as representative for the lavaged lung segment (Collie *et al.*, 2001; Reinhold *et al.*, 2005). However, the lavage of the cranial lung lobes segment is very important as these lobes are the most frequently affected by respiratory pathogens (Reinhold *et al.*, 2005; Došen *et al.*, 2007; Hennig-Pauka *et al.*, 2007). The BALF method has been widely discussed by Kiem and Schentag (2008). The large variation in antibiotic concentrations in ELF could be associated with BAL technique, which could be a source of errors. The tendency to overestimate the recovered volume of ELF also increases with increasing dwelling time with BAL technique. Studies in humans show a variable dwelling time of BAL from about three to more than 10 min. The most common method achieving an adequate recovery rate of rinsing fluid during BAL in pigs is the infusion of small fractions of rinsing fluid which are aspirated immediately (Turner-Warwick & Haslam, 1986; Reinhold *et al.*, 2005). In this study, the procedure of instillation and collection (BAL) was completed in <1 min (the average dwelling time 45 ± 10 sec), and the contact time between the instilled saline and the ELF was lower than the delay time between the beginning of instillation and the recovery of fluid. It is therefore possible that the fosfomycin concentration in the recovered ELF may be affected by a low diffusion of urea from the interstitial space and blood into the ELF. We consider that it did not markedly modify the results. In fact, the calculated extracellular lining fluid volumes of 0.56 ± 0.15 mL for a 21-mL lavage seem to be in the suitable range for this fluid (Rennard *et al.*, 1986; Marcy *et al.*, 1987; van Leengoed & Kamp, 1989; Feng *et al.*, 1992; Lamer *et al.*, 1993; Muller-Serieys *et al.*, 2001). Besides, Dargaville *et al.* (1996) have demonstrated that with short dwelling time (<1 min), urea is a valid marker of dilution in BAL fluids in normal, diseased, and recovering infant's lungs. The efficacy of antimicrobial agents against pulmonary infections depends on their local concentrations in the lung (Nix *et al.*, 1991; Toutain *et al.*, 2002; Kiem & Schentag, 2008). We found that the concentrations of fosfomycin were significantly lower in bronchial ELF than in serum at all sampling time points (Fig. 1).

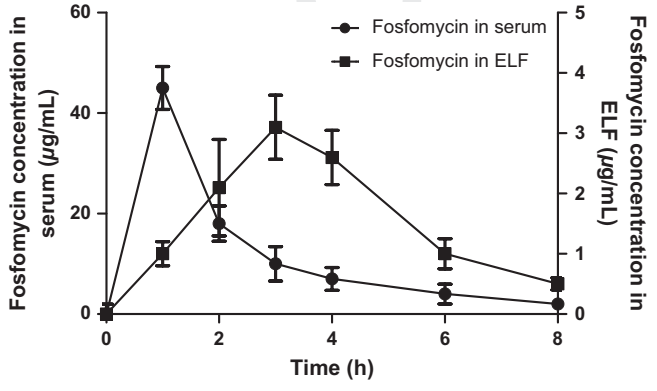

Fig. 1. The mean ± 1 SD concentrations of fosfomycin in serum and epithelial lining fluid collected after a single i.m. dose of 15 mg/kg b.w. in weaning piglets.

Table 1. Some pharmacokinetic parameters of fosfomycin in serum and epithelial lining fluid (ELF) obtained after a single i.m. dose of 15 mg/kg b.w. in weaning piglets

Parameters	Serum	ELF
AUC_{0-8} µg·h/mL	98.70 ± 2.70*	12.37 ± 1.43
C_{max} µg/mL	45.00 ± 2.51†	3.10 ± 0.95
T_{max} h	1.00 ± 0.00‡	2.58 ± 0.49
$t½$ h	1.98 ± 0.15§	1.33 ± 0.37

*P value was 0.0001; †P value was 0.0001; ‡P value was 0.0005; §P value was 0.0173.

Some comparative pharmacokinetic data considered in ELF and serum showed significantly lower values than that in plasma ($P < 0.05$) (Table 1). Fosfomycin concentrations in ELF were 12.1% of those of serum. These values are similar to those observed with other hydrophilic β-lactam and aminoside antibiotics such as gentamicin in humans (Honeybourne, 1994; Kiem & Schentag, 2008). Several mechanisms may limit the penetration of fosfomycin into ELF: (i) The anatomy of the blood–alveolar barrier is composed of two membranes that are separated by interstitial fluid. The alveolar epithelial cells are tightly opposed by numerous zonulae occludens that may not be completely penetrable by nonlipophilic (poor lipophilicity) compounds, such as fosfomycin, and therefore make difficult the passage of such antibiotic molecule (Baldwin *et al.*, 1992; Feng *et al.*, 1992; Taylor *et al.*, 1956;.; Theodore *et al.*,1975), (ii) The degree of fosfomycin ionization at the plasmatic pH may further restrict the transport of this antibiotic into ELF (Baldwin *et al.*, 1992; Lamer *et al.*, 1993). (iii) The free fraction (unbound) of the antibiotic can only reach equilibrium between serum and interstitial fluid. Binding to proteins may affect the antibiotic concentrations reached in the ELF (Kiem & Schentag, 2008). However, it is unlikely that the concentrations of fosfomycin collected in ELF can be influenced by the binding to proteins since the protein and cellular binding of fosfomycin is negligible (Kirby, 1977). In addition, it distributes marginally into cells and predominantly into the extracellular space fluid (Kestle & Kirby, 1969; Popovic *et al.*, 2009). The degree of penetration of fosfomycin into the ELF was determined by comparing the AUC_{0-8} of ELF with the AUC_{0-8} of serum. The ratio of fosfomycin AUC in ELF compared to AUC in serum (AUC_{ELF}/AUC_{serum}) was 0.15 ± 0.02. Similar values were observed for different β-lactams in ELF of humans after oral administration (range 0.13–0.15) and lower than amoxicillin in pig bronchial mucosa ($AUC_{mucosa}/AUC_{plasma}$: 0.37) after i.v. administration (AgersØ & Friis, 1998). This last variability with amoxicillin may partly be explained by the routes of administration. The intravenous administration of amoxicillin leads to a high concentration gradient between plasma and bronchial mucosa which facilitates drug penetration (AgersØ & Friis, 1998). The C_{max} in serum and ELF were 45.00 ± 2.5 1 and 3.10 ± 0.95 1, respectively. These concentrations were achieved at different times (T_{max} in serum and ELF: 1.00 ± 0.00 were 2.58 ± 0.49 h, respectively). Similar results were observed with cefdinir in plasma and in blister fluid (Richer

et al., 1995). The influence of a poor lipophilicity and diffusibility of fosfomycin could explain the slow and low capacity of this antibiotic for penetrating through the alveolar epithelial cells. Joukhadar *et al.* (2003) showed that penetration of fosfomycin concentrations in muscle interstitium was lower than that in plasma achieved at different times (T_{max} in muscle interstitium: 1 h and T_{max} in plasma: 0.4 ± 0.1 h). A similar result was observed in bone and peripheral soft tissue after perfusion of fosfomycin in diabetic patients presenting bacterial foot infection (Schintler *et al.*, 2009). The mean AUC fosfomycin in plasma and skeletal muscle was 673 (459–1108) and 477 (226–860) mg·h/L, respectively, and interstitial maximum concentrations were lower than plasma values (Schintler *et al.*, 2009). Optimal bacterial killing by fosfomycin will be achieved when the time period exceeding the MIC for the relevant pathogen (t > MIC) is maximized % T > MIC (Sumano *et al.*, 2007; Gutierrez *et al.*, 2009; Popovic *et al.*, 2009; McKellar *et al.*, 2004). Fosfomycin is considered a time-dependent antibiotic. Different authors have determined a fosfomycin MIC_{90} for *Streptococcus* sp. of 0.25 μg/mL (Fernandez *et al.*, 1995; Sumano *et al.*, 2007). The *Streptococcus* sp. is considered an important secondary agent in respiratory diseases of pigs (Gardner & Hird, 1990; Galina *et al.*, 1994; Done & Paton, 1995; Christensen *et al.*, 1999; Thanawongnuwech *et al.*, 2000; Carr, 2001; Cloutier *et al.*, 2003; Došen *et al.*, 2007). The fosfomycin concentrations in ELF were above the MIC_{90} value for *Streptococcus* during more than 8 h post i.m. administration of 15 mg/kg in weaning piglets. However, additional studies should be carried out in the lungs of infected pigs.

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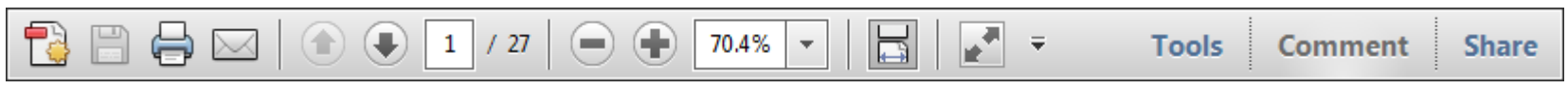

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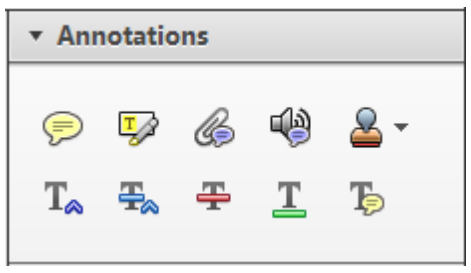

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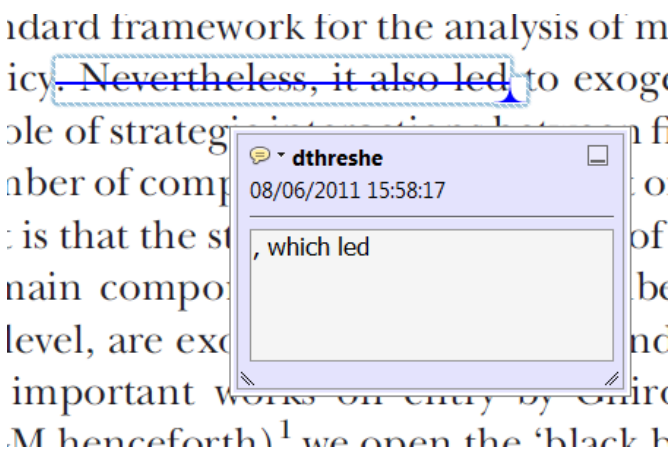

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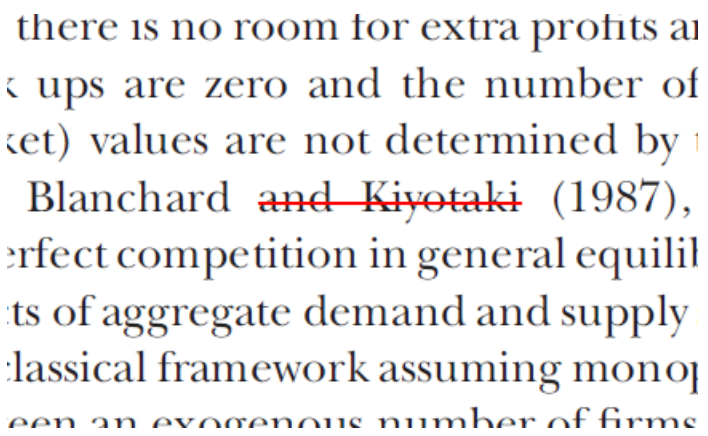

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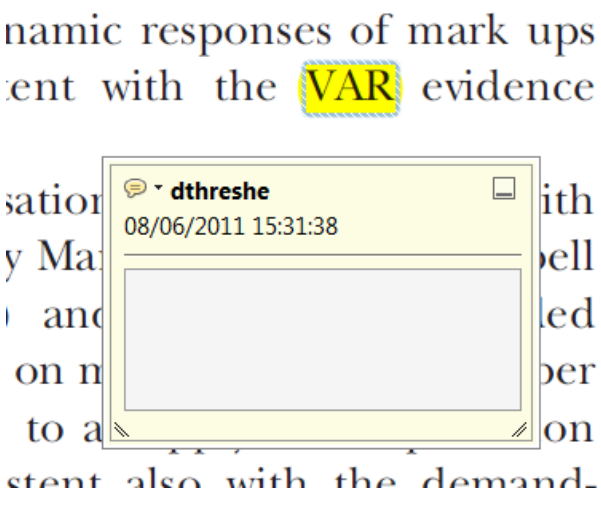

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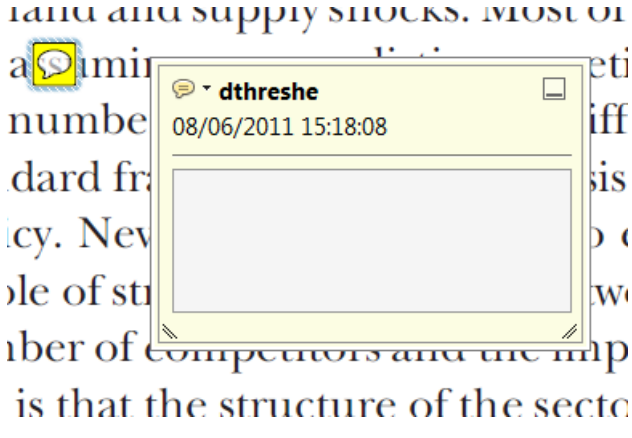

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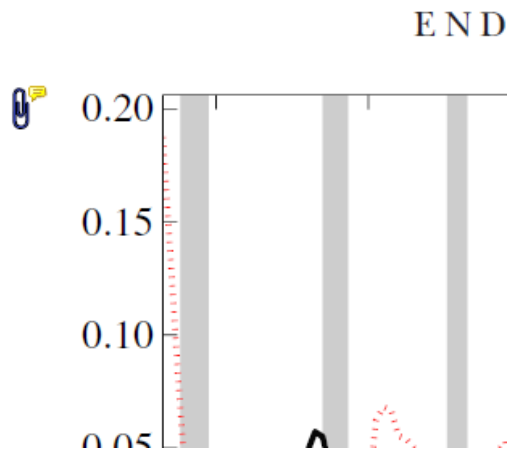
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

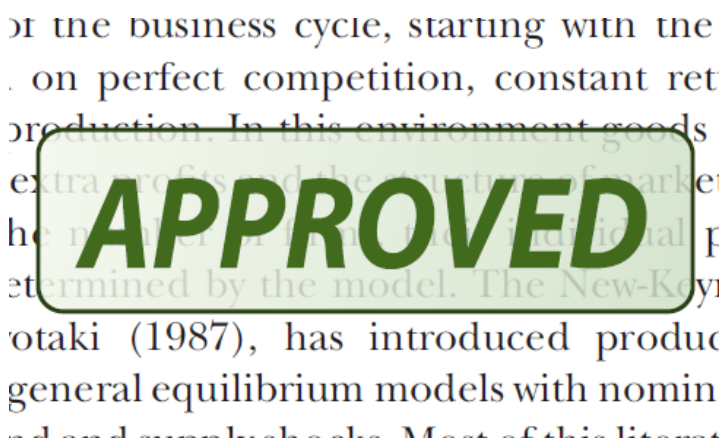


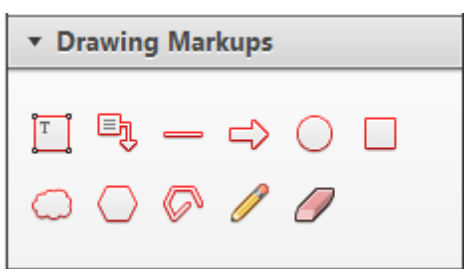
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

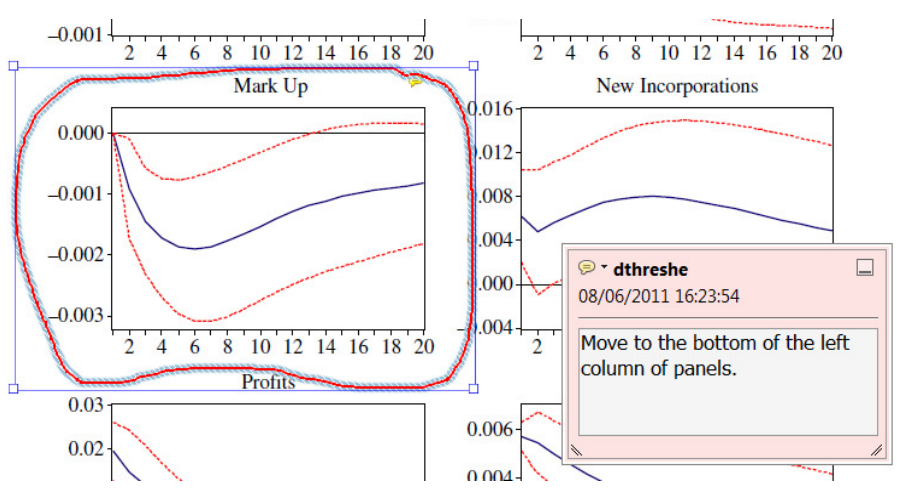
- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).





7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.

For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: