Case report: use of a plastic-coated catheter for transtracheal wash in 37 dairy calves affected by respiratory disease complex

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SUMMARY

Nasal or nasopharyngeal swabs are often employed for etiological diagnoses of bovine respiratory disease complex (BRDC), but transtracheal wash (TTW) can provide samples to assess the microbiological status of the lower respiratory tract because there is no contamination of the microorganisms from the upper respiratory airways. In large animals, a TTW is performed by means of angiocatheter or trocar, where a rubber tube or urinary catheter can be inserted. Although sterile instruments are commonly recommended, there is little practical information on the available tools for TTW. This case series reports the use of a disposable sterile single lumen polyurethane and plastic-coated venous catheter (Cavafix® Certo with Splittocan®, BBraun, Milan, Italy) to perform TTW in dairy calves affected by BRCD. Thirty-seven Holstein-Friesian calves with BRDC, admitted to the veterinary teaching hospital, underwent a TTW. After sedation of the calf, the disposable catheter was inserted into the tracheal lumen and 20 mL of sterile saline solution was injected and re-aspirated.

The procedure was always well tolerated and allowed to isolate the pathogenic microorganisms in 30 cases (81%). A single pathogenic microorganism was isolated in 23 cases, while in 7 cases, multiple isolations were performed. The most frequently isolated pathogens were *Pasteurella multocida* (13 cases, 35.14%) and *Mycoplasma bovis* (11 cases, 29.76%). Parainfluenza type 3 virus (PI-3) and bovine respiratory syncytial virus (BRSV) were both isolated in 4 cases. In 7 calves, already treated with antibiotics before hospitalization, the samples resulted sterile. In cattle the most employed tools to perform TTW are represented by urinary catheters or polyethylene tubes but they are not equipped with protective devices, that preserve sterility during the procedure. The characteristic plastic sheath covering Cavafix® Certo catheter avoids contamination during the entire procedure, increasing thereby the asepsis level of this technique. The use of Cavafix® Certo catheter is a simple, effective, and safe technique that can help to maintain asepsis throughout the procedure, especially in field conditions.

KEY WORDS

Bovine respiratory disease complex, calves, diagnostic techniques, transtracheal wash.

INTRODUCTION

Determining the etiology of bovine respiratory disease complex (BRDC) is a challenge for practitioners[1]. Microbiological tests are needed to provide an overview of the herd and to establish the most appropriate antibiotic therapy or correct vaccination plans. Nasal or nasopharyngeal swabs are often used for etiological diagnoses of BRDC, but a transtracheal wash (TTW) can provide samples for a broader diagnostic approach than nasopharyngeal swabs[2]. In fact, a TTW is optimal for assessing the microbiological status of the lower respiratory tract as there is no contamination of the microorganisms from the upper respiratory airways[3], which are not always genetically correlated to those found deeper[1].

In large animals, a TTW entails using angiocatheters or trocars, inside which rubber tubes or urinary catheters can be inserted[1,3]. Although sterile instruments are commonly recommended[4], there is little practical information on the tools that can be used during a TTW.

In this case series, we present our experience in using a disposable sterile single lumen polyurethane and plastic-coated venous catheter (Cavafix® Certo with Splittocan®, BBraun, Milan, Italy), in performing TTW in dairy calves affected by BRCD.

CASES PRESENTATION

Between January and December 2015, 37 Holstein Friesian calves (4 male and 33 female) were admitted to the Veterinary Teaching Hospital of the University of Milan because affected by BRDC. Twenty-four calves (65%) came from farms with a recent outbreak of BRDC and were not subjected to therapy before admission. The remaining 13 patients (35%) were subjected to antimicrobial therapy before admission because

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they came from farms with a history of recurrent or refractory episodes of BRDC. At the time of hospitalization, each patient was weighed and given a full clinical examination, as described by Pravettoni et al.[5]. Calves were also scored using the Wisconsin calf respiratory scoring chart (CRSC)[6]. Patient characteristics and clinical signs are summarized in Table 1.
To identify the pathogens involved in the disease, each calf was subjected to TTW. TTW was performed using Cavafix® Certo with Splittocan® (BBraun, Milan, Italy), a disposable plastic-coated venous catheter. Cavafix® Certo is a medium-long-term, radiopaque, single lumen, polyurethane, 16 G central venous catheter, coated by a transparent plastic sheath, to guarantee sterility during its use, available in different lengths (32, 50, 70 cm), employed in humans for vena cava catheterization (Palmieri et al., 2012). We used a 32-cm catheter which allowed to take samples from the tracheal bifurcation of

Table 1 - Clinical data of 37 calves affected by respiratory disease complex enrolled in this case report.

Calf	Age (days)	Bodyweight (kg)	Wisconsin calf respiratory score*				
			Nasal discharge	Eye/ear	Cough	°C	Total
1	86	71	1	1	2	3	7
2	39	47	2	1	2	2	8
3	42	46	2	2	3	3	10
4	34	39	2	3	3	2	10
5	53	49	1	1	1	3	6
6	34	48	1	1	1	3	6
7	90	78	2	3	2	3	10
8	35	44	3	3	3	2	11
9	71	59	1	1	1	2	5
10	109	82	3	1	3	3	10
11	78	56	1	2	2	3	8
12	43	49	1	1	3	3	8
13	55	53	1	0	1	3	5
14	122	111	2	2	1	0	5
15	115	97	3	0	3	3	9
16	69	62	3	2	3	1	9
17	46	51	2	1	1	3	7
18	74	81	3	1	1	3	8
19	79	77	1	1	2	3	7
20	120	99	1	1	3	3	8
21	195	133	2	2	2	0	6
22	137	99	1	3	3	1	8
23	174	141	2	1	1	3	7
24	50	49	1	1	1	3	6
25	36	51	1	1	3	3	8
26	43	45	3	1	3	2	9
27	61	77	3	1	2	1	7
28	58	73	1	1	1	2	5
29	62	58	3	3	2	1	9
30	35	49	3	2	3	3	11
31	56	61	1	0	2	2	5
32	40	55	2	1	2	3	8
33	90	74	3	2	1	1	7
34	122	93	2	2	2	2	8
35	90	66	1	0	2	3	6
36	62	51	2	0	2	3	7
37	45	40	2	1	3	3	10

*Wisconsin calf respiratory scores were evaluated according to McGuirk, 2008[6]. Eye discharge and ear position were considered together, recording the highest score as a single value. Nasal discharge score and eye discharge and ear score were assigned during the clinical examination. Occasional or repeated spontaneous coughing was also noted during clinical examination, giving the calf 2 or 3 points respectively. In calves that did not show spontaneous coughing, cough induction was performed by tracheal compression and 1 or 2 points were assigned for single or repeated induced cough respectively. Rectal temperature (°C) was then recorded, and the clinical examination was completed. Total respiratory score of 5 or more were considered to have respiratory disease.

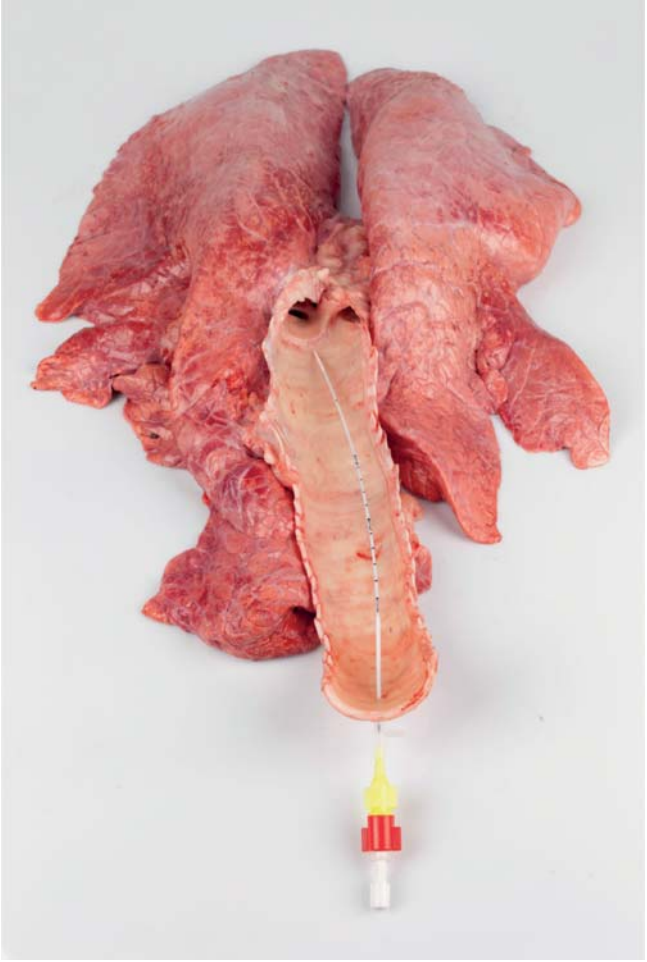

Figure 1 - Simulation of a transtracheal wash performed in an anatomical preparation of a young bull collected in a slaughterhouse. The end of the Cavafix® Certo catheter, 32 cm long, inserted into the tracheal lumen, is placed near the bronchial bifurcation.

calves up to 195 days of age (Figure 1).

After intravenous sedation with 0.2 mg/kg xylazine (Rompun®, Bayer AG, Leverkusen, Germany), calves were placed in sternal recumbency with head lift and neck extended by an assistant. A skin area of 5 x 5 cm was shaved on the ventral surface of the middle third of the neck, along the tracheal course. After surgical scrub with 70% alcohol and povidone-iodine, local anesthesia was performed by subcutaneous infiltration of 2 mL 2% procaine hydrochloride (Procamidor; Izo s.r.l., Brescia, Italy). The trachea was immobilized between the fingers and a 1-cm long longitudinal skin incision was made in the midline. A 14 G x 5 cm cannula needle, contained in the Cavafix® Certo kit, was inserted into the trachea between two cartilaginous rings (Figure 2a). The cannula was completely inserted into the tracheal lumen by moving it ventrally until the needle grip makes contact with the incised skin. After removing the steel needle, a 32-cm long Cavafix® Certo catheter was joined to the cannula through its yellow coupling piece and moved forward into the tracheal lumen, pushing it through the outer protective plastic sheath (Figure 2b). After placing the entire catheter into the tracheal lumen, the protective plastic sheath was torn by holding the red coupling piece between the fingers and the radiopaque mandrel of the catheter was extracted. During this procedure, the Luer Lock closure of the catheter was carefully kept inside the red coupling piece.

Twenty mL of sterile saline solution was injected with a sterile syringe and re-aspirated from the tracheal lumen, using the same syringe (Figure 2c and 2d). The procedure was considered satisfactory if at least 5 mL of washing fluid was re-aspirated. During this procedure, the head of the calf was gradually returned to a horizontal position. At the end of the procedure, the catheter and the cannula were removed. A single surgical stitch was applied on the incision site, using a non-absorbable polyamide suture thread which was removed 10 days after the procedure. The time spent to perform the whole procedure ranged between 10 and 15 minutes. After TTW, calves were housed in individual brick pens in an indoor 20-place stall. Although all the calves coughed during catheter insertion, the procedure was always well tolerated, and upon waking from sedation no animal showed signs of pain or further respiratory distress. There were no cases of post-procedural complications. In 34 calves (91%), the use of Cavafix® Certo enabled a sufficient amount of washing fluid (> 5 mL) to be harvested. Only 3 (9%) of the 37 patients required a further injection of 20 mL of saline solution due to the re-aspiration of a too small quantities of washing-fluid.

The sample was transferred into a sterile single-use tube, immediately taken to the laboratory, and cultured on blood agar and PCR for *Mycoplasma* spp., parainfluenza type 3 virus (PI-3), bovine respiratory syncytial virus (BRSV), and bovine herpes virus-1 (BHV-1). Antibiotic assay was performed according to the Kirby-Bauer technique. The amount of collected fluid allowed to carry out both bacteriological and virological tests and the research for *Mycoplasma* spp. Transtracheal airway washes allowed isolation of pathogenic microorganisms in 30 cases (81%). A single pathogenic microorganism was isolated in 23 cases, while in 7 cases, multiple isolations were performed. The most frequently isolated pathogens were *Pasteurella multocida* (13 cases, 35.14%) and *Mycoplasma bovis* (11 cases, 29.76%). In 7 calves, already treated with antibiotics before the hospitalization, the samples resulted sterile. The results of microbiological tests and antibiotic assays are summarised in Table 2.

Calves were treated with a single subcutaneous injection of tilmicosin at a dose of 10 mg/kg (Micotil 300; Lilly Italia; Sesto Fiorentino; Italy) before the results of the microbiological assay were known. Anti-inflammatory and supportive therapies were performed according to the standard approach of undifferentiated BRDC in cattle[7]. No change of antibiotics was performed after the results of microbiological assay, because no cases were found to be insensitive to treatment with tilmicosin. After therapy, only 7 calves (19%) died during the hospitalization, while the other 30 calves were discharged from the hospital with a normal hydration and vigor score, good appetite, and a respiratory score <5. The average length of hospitalization was 17.4 ± 4.6 days.

DISCUSSION

Our results show that Cavafix® Certo is an effective tool to perform TTW in dairy calves in a simple and rapid way. Cough during insertion of the transtracheal catheter represent a normal and transient consequence of TTW[8]. The 32-cm catheter employed, allowed to take samples from the tracheal bifurcation, with precise control in terms of both directing and placing the catheter into the tracheal lumen.

In three calves, after the 20-mL saline injection it was not pos-

Table 2 - Results of microbiological exams on transtracheal aspirates and related antibiotic assay. The most frequently isolated pathogens were *Pasteurella multocida* (13 cases, 35.14%) and *Mycoplasma bovis* (11 cases, 29.76%). In 8 patients (21.62%), other species of *Mycoplasma* were identified. Four samples (10.81%) were positive for bovine respiratory syncytial virus (BRSV), and 4 (10.81%) for parainfluenza type 3 virus (PI-3). In 7 (19%) cases no pathogens were isolated.

Calf	Antimicrobial therapy before admission	Microbiological results	Sensitive	Intermediate	Resistant
1	No	*P. multocida*	*Ax, Am, Cf, En, Gn, St, Tc*		
2	No	*Mycoplasma* spp.			
3	Yes	*T. pyogenes, Mycoplasma* spp.	*Cq, Cf, Dx, St, Tc*	*An, Am, Dn, En, Mr, Pn*	*Fl, Gn, Kn, Sp, Tt, Tf, Tm, Tl*
4	Yes	Sterile			
5	No	*P. multocida*, *M. bovis*	*Ax, Am, Cf, En, Gn, St, Tc*		
6	No	*M. bovis*			
7	No	*P. multocida*	*An, Am, Cq, Cf, Dn, Dx, En, Fl, Gn, Kn, Mr, Pn, Sp, Tc, Tt, Tf, Tm, Tl*		
8	No	*T. pyogenes, M. bovis*	*An, Am, Cq, Cf, Fl, Sp, Tf, St*	*En, Kn, Mr, Pn*	*Dn, Dx, Gn, Tt, Tm, Tl*
9	No	*M. haemolytica, Mycoplasma* spp.	*An, Am, Cq, Cf, Dn, En, Fl, Gn, Kn, Mr, Sp, Tc,Tt, Tf, Tm, Tl, St*	*Dx*	*Pn*
10	No	PI-3, *P. multocida*	*An, Am, Cq, Cf, Dn, Dx, En, Fl, Gn, Kn, Mr, Pn, Sp, Tc, Tt, Tf, Tm, St*		*Tl*
11	No	*M. bovis*			
12	No	*M. bovis*			
13	No	*P. multocida*	*Tc*	*An, Cf, Dn, Dx, En, Fl, Gn, Tf*	*Ac, Am, Cq, Kn, Mr, Pn, Sp, Tt, Tm, Tl, St*
14	No	*P. multocida*	*An, Am, Cq, Cf, Dn, Dx, En, Fl, Gn, Kn, Mr, Sp, Tc, Tt, Tf, Tm, Tl, St*	*Pn*	
15	No	BRSV			
16	No	BRSV			
17	Yes	*Mycoplasma* spp.			
18	No	*Mycoplasma* spp.			
19	Yes	*M. bovis*			
20	No	*P. multocida*	*An, Am, Cq, Cf, Dn, Dx, En, Fl, Gn, Kn, Mr, Pn, Sp, Tc, Tt, Tm, Tl, St*		
21	No	*P. multocida*	*An, Am, Cq, Cf, Dn, Dx, En, Fl, Gn, Kn, Mr, Pn, Sp, Tc, Tt, Tm, Tl, St*		
22	Yes	Sterile			
23	No	BRSV			
24	Yes	Sterile			
25	Yes	Sterile			
26	No	BRSV, *P. multocida*	Am, Cq, Cf, Dn, Dx, En, Fl, Gn, Kn, Mr, Pn, Sp, Tc, Tt, Tl, St		
27	No	*M. bovis*			
28	Yes	Sterile			
29	Yes	Sterile			
30	No	*P. multocida*, *M. bovis*	Tc	Fl, Tt	An, Am, Cq, Cf, Dn, En, Mr, Pn, Sp, Tl, St
31	Yes	PI-3, *P. multocida*	Ac, Am, Cp, Cf, En, Fl, Gn, Kn, Pn, Tc	Cl, Tt	St
32	Yes	Sterile			
33	No	PI-3, *P. multocida*, *M. haemolytica*	*P. multocida:* Ac, Am, Cp, Cf, En, Fl, Gn, Kn, Pn, Tt, Tc, St *M. haemolytica*: Ac, Cp, Cf, En, Fl, Gn, Kn, Tt, Tc, St		P. multocida: Cl M. haemolytica: Am, Cl, Pn
34	Yes	PI-3, *P. multocida*	Ac, Am, Cp, Cf, En, Fl, Gn, Kn, Pn, Tt, Tc, St		Cl
35	No	*Mycoplasma spp., T. pyogenes, M. bovis*	An, Cq, Cf, Dn, Dx, En, Fl, Gn, Kn, Mr, Pn, Sp, Tt, Tm, Tc,Tl, St		Am
36	Yes	*Mycoplasma spp., T. pyogenes, M. bovis*	An, Cf, Dn, Dx, En, Fl, Gn, Kn, Mr, Pn, Sp, Tc, Tt, Tm, Tl, St	Am, Cq	
37	No	*Mycoplasma spp., T. pyogenes, M. bovis*	An, Am, Cq, Cf, Dn, Dx, Fl, Gn, Kn, Mr, Pn, Sp, Tc, Tt, Tm, Tl, St	En	

Ac = Amoxicillin + Clavulanic acid, Am = Ampicillin, An = Paromomycin, Cf = Ceftiofur, Cq = Cefquinome, Dn = Danofloxacin, Dx = Doxycycline, En = Enrofloxacin, Fl = Florfenicol, Gn = Gentamicin, Kn = Kanamycin, Mr = Marbofloxacin, Pn = Penicillin, Sp = Spiramycin, St = Sulfonamides + Trimethoprim, Tc = Tilmicosin, Tf = Thiamphenicol, Tl = Tylosin, Tm = Tiamulin, Tt = Tetracycline.

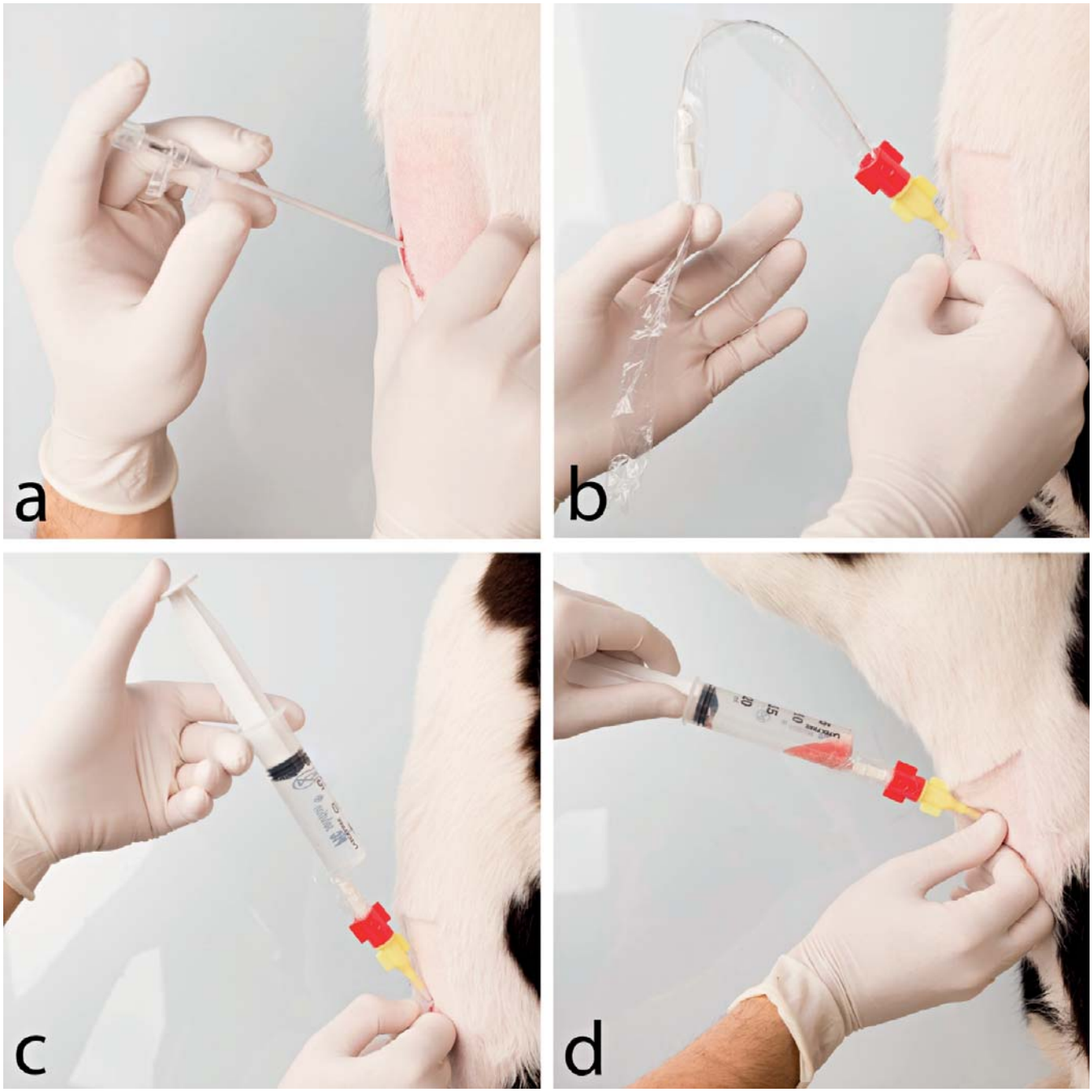

Figure 2 - After the complete insertion of the cannula into tracheal lumen (a), the entire catheter can be placed into the tracheal lumen by pushing it through the outer protective plastic sheath (b). Twenty mL of sterile saline solution was injected with a sterile syringe (c) and re-aspirated from the tracheal lumen (d).

sible to re-aspirate an adequate amount of flushing fluid. This is a common condition during TTW and can be easily fixed by a further injection of 20 mL of saline solution[4]. In large animals, complications such as subcutaneous emphysema, local cellulitis or loss of the catheter into the tracheal lumen have been described[4]. In our experience, no complications were recorded. Thanks to the two interlocking pieces (the red and yellow pieces) it is almost impossible to lose the Cavafix® Certo inside the lumen. The kit is also packaged in a sterile box and only the translucent angiocatheter hub can come into contact with the incised skin, thus preventing contamination of the incision area. The whole procedure was easy to perform, thanks to the presence of an assistant who kept the calf's head extended on the neck.

In cattle, urinary catheters or polyethylene tubes are used almost exclusively to perform TTW[1,3], but they are not equipped with protective devices, which preserve sterility during the procedure. The characteristic plastic sheath that covers the Cavafix® Certo catheter avoids contamination during the entire procedure because the catheter that will be placed into the tracheal lumen, never encounters the external environment, increasing thereby the level of asepsis of this technique. The results shown in Table 2 confirm this interpretation. In fact, in untreated calves only the causative agents of BRDC were isolated, while in the 7 calves treated with antimicrobials, the procedure was associated with complete sterility of the washing fluid.

In affected calves, TTW enables pathogens to be isolated[3,8,9]. Our results showed that the TTW allowed to isolate pathogens in 30/37 cases. Negative results occurred only in calves already treat-

ed with antimicrobials before hospitalization (7/13 calves). The results of some bacterial culture tests are reported to be influenced by previous antibiotic therapy[1]. This result is clinically relevant: when TTW is carried out in a farm to identify pathogens involved in the BRDC and to choose the most appropriate treatment and vaccination protocol, the sample size should be constituted by 6 calves, as reported by Gorden & Plummer (2010) and our suggestion is to choose calves with active BRDC but not already treated, in order to limit false negatives.

CONCLUSION

In conclusion, our study results reinforce the few data available on TTWs performed in sterile conditions in cattle, thus showing that the use of Cavafix® Certo catheter is a simple, effective, and safe technique that can help to maintain asepsis throughout the procedure, especially in field conditions.

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