Original Article

Unravelling the productivity rate of selective media for Brucella strains

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Abstract

The aim of this study is to reveal the productivity rate of selective media for Brucella strains. Brucella selective media namely Farrell's medium, CITA, Modified Thayer-Martin, Jones-Morgan medium and one additional medium including erythritol and antibiotics were compared within the scope of productivity rate and colony growth in time. The comparison was carried out using 14 strains including Brucella abortus biovars, S19 vaccine strain, Brucella suis biovars and Brucella ovis. Erythritol addition showed a positive effect on colony growth in time. In addition to differences between the productivity rates of the media; there was also some variation in the colony size in time among different media. Modified Thayer Martin medium presents the highest productivity rate for the majority of the strains used. Achieving successful isolation depends on the productivity rate and medium's capacity for inhibiting the contaminant microorganism. The data related to both productivity rate and colony size growth could be beneficial to increasing isolation chance through the selection of the most appropriate medium depending on the target Brucella species and biovar in the sample.

Keywords: brucella, isolation, productivity rate, selective medium

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Introduction

Brucellosis, as a common zoonosis, causes health issues for both animals and humans all over the world (Boschiroli *et al.,* 2001; Yumuk and O'Callaghan, 2012; McDermott *et al.,* 2013; Ducrotoy *et al.,* 2014; Hou *et al.,* 2019; Wareth *et al.,* 2019). The causative agent of the disease is *Brucella* spp., which has spread over a huge area in the world, including more than 170 countries and regions (Hou *et al.,* 2019). More than 500,000 new human cases associated with brucellosis are estimated each year (Pappas *et al.,* 2006; Nicoletti, 2010). Brucellosis is considered to be a multiple-burden disease with a negative impact on humans, animals and wildlife (McDermott *et al.,* 2013). It causes health and economic problems, especially in endemic locations (Boschiroli *et al.,* 2001; Yumuk and O'Callaghan, 2012; Zhang *et al.,* 2018), such as the Mediterranean countries (Garin-Bastuji *et al.,* 1998; Banai, 2002; Zhang *et al.,* 2018; Wareth *et al.,* 2019). The major clinical manifestations of brucellosis in animals include late-term abortion, stillbirth, retained placenta, mastitis and metritis, which result in reproductive insufficiency and infertility (Garin-Bastuji *et al.,* 1998; OIE, 2018[a]; Wareth *et al.,* 2019; Jamil *et al.,* 2020).

Brucellosis in cattle is caused by biovars of *B. abortus* (Seleem *et al.,* 2010). In rams, *B.ovis* is the pathogen of the disease leading to epidimit. Abortions and infertility in sheep and neonatal mortality in lambs are rarely caused by *B.ovis* (OIE, 2018[b]). Sporadic infections due to *B.abortus* and *B.suis* in sheep and goats are occasionally reported. Swine brucellosis is related to *B.suis* biovar 1, 2, or 3 among 5 biovars (OIE, 2018[a]).

In the diagnosis of brucellosis, bacterial isolation is considered to be the unequivocal method (OIE, 2018[a]). *Brucella* bacteria, which are fastidious microorganisms requiring longer incubation periods can be found with contaminant microorganisms in samples such as abortion materials, placenta and milk. It has been reported that the media used for the bacteriological culture of brucellosis-suspected field samples can be contaminated with secondary microorganisms and these media can be turned into selective media that suppress the growth of contaminant microorganisms and ensure isolation success with the addition of antibiotics (Alton *et al.,* 1988; Stack *et al.,* 2002; Poester *et al.,* 2010). Because the number of contaminant organisms growing rapidly in the diagnostic material is high, a selective medium for *Brucella* spp. isolation is required (Moris, 1956; Marin *et al.,* 1996[a]; Stack *et al.,* 2002). There is a great variety of selective media types including different basal media, antibiotic mixture and concentration (Hornsby *et al.,* 2000). According to previous studies, every medium has a specific effect on *Brucella* species, its biovars and contaminants due to the media-related differences (Marin *et al.,* 1996[b]; Vicente *et al.,* 2014). 'W' medium, one of the first media used for *Brucella* isolation, was developed by Kuzdas and Morse in 1953 (Kuzdas and Morse, 1953). Following this, Jones-Morgan medium (JM); namely, the serum dextrose agar with antibiotics was prescribed by Jones and Morgan (1958) with some changes in W medium. In addition to these first attempts for developing a selective medium for *Brucella* spp, Farrell's medium (FM), Modified Thayer Martin medium (MTM) and CITA are the preferred selective media for *Brucella* isolation from field samples (OIE, 2018[a]). In a study by Karagul and Ikiz (2017), which compares the isolation and inhibition abilities of *Brucella* selective media in artificially inoculated organ samples by *Brucella* field strains; it was found that FM has the highest isolation percentage (92.1%) whereas CITA and MTM have very close isolation performance (88.2%) to FM. However, FM showed higher inhibition ability (80.4%) than CITA and MTM. In the South African context, the selective media FM, modified CITA and modified MTM for optimal isolation of *Brucella* spp. were investigated through *B.abortus* biovar 1 and biovar 2 as the most prevalent strains. Ledwaba *et al.* (2020), identified modified CITA medium as the optimum selective medium for *Brucella* spp. isolation whereas FM was found to inhibit the growth of fungal and bacterial contaminants. In another related study, which focused on the performance of *Brucella* selective media in ruminant abortion cases, selective media were evaluated based on their isolation rates and contaminant inhibition. FM and MTM showed the best isolation performance, respectively whereas FM and CITA provided higher contaminant inhibition than other media used (Karagul and Ikiz, 2018). Chemicals, particularly antibiotics added to selective media in order to inhibit the contaminants, may also affect the growth of *Brucella* species adversely and may decrease the isolation rate (Marin *et al.,* 1996[b]; Drancourt and Raoult, 2007).The aim of this study is to unravel the productivity rates of the selective media for *Brucella* isolation using different species and biovars with a compare-contrast approach.

Materials and Methods

Brucella reference strains provided from culture stocks of Pendik Veterinary Control Institute, *Brucella* Reference and Vaccine Production Laboratory were utilized in this study and the list of the strains is given in Table 1 below.

In order to prepare 1 L from each selective medium, the required basal medium for the selected media was weighed in a 2 L flask. It was later sterilized through autoclaving (121 °C ± 3 °C for 20 mins). When autoclaving was over, flasks including media were put into a water bath at the temperature of 50 °C. Depending on their contents, sterile new born calf sera and antibiotics were added to the media (Alton *et al.* 1988). Prepared medium was distributed to petri dishes in the amount of 23 ml and that provided minimum 3 mm thickness in petri dishes with a 90 mm diameter. To conduct sterility control, solidified media were incubated at 37 °C for 48 hours (ISO/TS 2009).

Natamycin and cycloheximid, which are included in JM medium, were replaced with amphotericin-B, which belongs to the same antifungal agent category as natamycin. Amphotericin-B is one of the antifungals recommended for the first isolation of *Mycobacterium* spp. (Drancourt and Raoult, 2007), *Campylobacter* spp. (Martin *et al.,* 2002), and *Brucella* spp. (De Miguel *et al.,* 2011) in selective media.

Table 1 List of reference strains

No	Strains	Biovar	No	Strains	Biovar
1	*B.abortus* 544	*B.abortus* bv 1	**8**	*B.suis*1330	*B.suis* bv1
2	*B.abortus*86/8/59	*B.abortus* bv2	**9**	*B.suis* Thomsen	*B.suis* bv2
3	*B.abortus*Tulya	*B.abortus* bv3	**10**	*B.suis* 686	*B.suis* bv 3
4	*B.abortus* 292	*B.abortus* bv4	**11**	*B.suis* 40	*B.suis* bv4
5	*B.abortus* B3196	*B.abortus* bv5	**12**	*B.suis*	*B.suis* bv5
6	*B.abortus* 870	*B.abortus* bv6	**13**	*B.ovis*63/290	-
7	*B.abortus* C68	*B.abortus* bv9	**14**	*B.abortus*S19	-

Four different selective media; namely, FM, MTM, CITA, JM and one basal medium, which is TSA, were used in this study. In order to extend the productiveness of the results, a potential *Brucella* medium as a test medium (TM) was also included. The content of the FM, CITA, MTM, JM and TM as selective media are listed in Table 2 (Jones and Morgan, 1958; Farrell, 1974; Marin *et al.*,1996[a]; De Miguel *et al.,* 2011).

Table 2 The contents of the media used

Content	FM	CITA	MTM	JM	TM
Basal medium	BMB-CS	BAB-CS	GC-H	SDA-CS	TSA-CS
Bacitracin (IU/liter)	25,000	-	-	25,000	-
Polymyxin (IU/liter)	5,000	-	-	6,000	6,000
Nalidixicacid (mg/liter)	5	-	-	-	-
Amphotericin-B (mg/liter)	-	4	2.5	4	4
Natamycin (mg/liter)	50	-	-	-	-
Nitrofurantain (mg/liter)	-	10	10	-	10
Vancomycin (mg/liter)	20	20	3	-	20
Colistin (mg/liter)	-	7.5	7.5	-	-
Nystatin (IU/liter)	100,000	100,000	100,000	-	-
Erythritol (g/liter)	-	-	-	-	1

BMB-CS: *Brucella* medium base with calf sera
BAB-CS: Blood agar base with calf sera
GC-H: GC agar base with hemoglobin
SDA-CS: Serum dextrose agar with calf sera
TSA-CS: Tryptic soy agar with calf sera

Lyophilized reference strains were reconstituted with distilled water and inoculated to TSA medium. The turbidity of the suspensions prepared from harvested colonies on solid medium was measured by spectrophotometer. Depending on the turbidity results, serial dilutions of the suspension were inoculated to TSA in order to determine bacteria count (cfu/ml). At the end of the incubation period, each colony was counted by colony counter and the *Brucella* concentration (cfu/ml) for each suspension was calculated (Jensen and Halling, 2010; Ferrira *et al.,* 2012). 100 microliters from these suspensions was inoculated to each of the two petri dishes of selective medium and the basal medium (Stack *et al.,* 2002). If the results of the 2 petri dishes were not close to each other, inoculation was repeated. If the results of one of the selective media was fairly higher or lower for one of the strains; the experiment for this strain was repeated in all the media to confirm the previous result.

We have incubated the media at 37 °C with 5-10% CO2 for 5-7 days and we have observed the media twice a day in order to determine the bacterial growth with the naked eye. For the identification of *Brucella* spp. from the growing colonies, macroscopic morphology of the colonies, gram staining, urease and oxidase activity, agglutination with polyvalent *Brucella* antisera and bacteriophage sensitivity were taken into consideration. Biovar identification of isolated strains was conducted through phage lysis, agglutination with anti-A, anti-M monospecific sera, CO2 requirement, H_2S production, and growth characteristic in media including thionin and basic fuchsin. Additionally, the growth characteristics of penicillin medium, streptomycin medium and erythritol medium were examined in order to confirm S19 vaccine strain (Alton *et al*., 1988; OIE, 2018[a]). *Brucella* colonies were counted and recorded at the end of the incubation period. The diameter of the recognizable colonies was measured from outside of the petri dishes by calipers. Colony count results, diameter measurements and the day on which colonies were visible via the naked eye were listed. The productivity rate (PR%) of the media in accordance with TSA medium was calculated (Her *et al.,* 2010). In order to get the productivity rate, the formula (%PR= Colony count on working media/ colony count on basal medium × 100) from ISO standard was used (ISO/TS, 2009). The results were analyzed with Pearson's chi-square test in SPSS 18.0. For the analysis, the media having the highest productivity rate were analyzed in comparison with the other media and statistically significant results were revealed for the ones having the p values smaller than .05.

Results

The colony growth of the *Brucella abortus* strains during the incubation period on selective media and the PR percentages regarding the total counted colony number at the end of the incubation are given in Table 3.

Table 3 Productivity rate of the media for *B.abortus (B.A.)* strains and the rankings of the strains by colony diameter

Strain	MEDIUM	Colony size (mm) on 4th-5th days	Ranking (colony size)	PR %	Strain	MEDIUM	Colony size (mm) on 4th day	Ranking (colony size)	PR%
B.A. bv1	TSA	> 1	1.	REF.	*B.A.* bv5	TSA	> 1	1.	REF.
	MTM	<1	4.	89		MTM	> 1	1.	96
	JM	≥ 1	2.	90		JM	≤ 1	2.	77
	FM	≥ 1	2.	82		FM	< 1	3.	76
	TM	≤ 1	3.	81		TM	> 1	1.	94
	CITA	≤ 1	3.	67		CITA	> 1	1.	95
B.A. bv2	TSA	> 1	1.	REF.	*B.A.* bv6	TSA	≥ 1	1.	REF.
	MTM	≥ 1	2.	62		MTM	≥ 1	1.	93
	JM	≤ 1	3.	9		JM	≤ 1	2.	84
	FM	> 1	2.	89		FM	≤ 1	2.	84
	TM	> 1	1.	11		TM	≥ 1	1.	94
	CITA	≤ 1	3.	8		CITA	≥ 1	1.	92
B.A. bv3	TSA	> 1	1.	REF.	*B.A.* bv9	TSA	> 1	1.	REF.
	MTM	≥ 1	2.	88		MTM	≤ 1	4.	98
	JM	≤ 1	3.	82		JM	≥ 1	3.	81
	FM	≥ 1	2.	83		FM	≥1	3.	89
	TM	> 1	1.	80		TM	> 1	1.	86
	CITA	≤ 1	3.	96		CITA	≥ 1	3.	96
B.A. bv4	TSA	> 1	1.	REF	S-19	TSA	≤ 2	2.	REF.
	MTM	≤ 0,5	4.	88		MTM	≥ 1	4.	97
	JM	≥ 1	2.	83		JM	> 2	1.	96
	FM	≤ 1	3.	77		FM	≤ 2	3.	96
	TM	≤ 0,5	4.	84		TM	0	6.	0
	CITA	≤ 0,5	4.	74		CITA	≤ 1	5.	85

As summarized in Table-3 above, CITA, JM, TM media have lower PR results for *B.abortus* bv2 unlike the other *B.abortus* biovars. There is no growth obtained from TM for S19 vaccine strain and therefore the PR is zero. Apart from these findings, all the media showed PR higher than 62% for *B.abortus* biovars. The average PR of *B.abortus* biovars for each medium according to total colony count is listed in Table 4.

Table 4 The average productivity rate of media for *B.abortus* biovars and S19 vaccine strain

Medium	PR%
MTM	88.9
FARREL	84.5
CITA	76.6
JM	75.25
TM	66.25

All these calculations were carried out regarding total colony counts on TSA medium. For TSA medium, the average colony count was found to be 169 colony forming units (CFU). In Table 5, only the statistically significant results of PR for *B.abortus* biovars are listed.

Table 5 The statistical results of the average productivity rate of media for *B.abortus* strains

Chi-Square Tests	PR	
Pearson Chi-Square	X^2	P value
FM & JM	4.713	.030
FM& TM	15.347	.000
MTM & JM	10.582	.001
MTM & CITA	8.325	.004
MTM & TM	24.511	.000
CITA & TM	4.714	.030

FM and MTM, which have higher PR than the other media, also showed a P-value lower than 0.05, which indicates that the differences are statistically significant. The differences between FM and MTM and also FM and CITA medium are not statistically significant.

The colony growth of *B.suis* biovars and *B.ovis* strain during the incubation period on selective media and the PR percentages regarding the total counted colony number at the end of the incubation are shown in Table 6.

Table 6 Productivity rate of the media for *B.suis and B.ovis* strains and the rankings of the strains by colony diameter on media

Strain	MEDIUM	Colony size (mm) on 4th-6th days	Ranking (colony size)	PR%	Strain	MEDIUM	Colony size (mm) on 4th day	Ranking (colony size)	PR%
B.suis bv1	TSA	> 1	2.	REF	*B.suis* bv5	TSA	> 2	1.	REF
	MTM	≤ 1	4.	92		MTM	≤ 2	2.	85
	JM	≥ 0.5	5.	86		JM	≤ 1	3.	66
	FM	a	6.	66		FM	< 1	4.	86
	TM	≤ 2	1.	95		TM	> 2	1.	94
	CITA	≥ 1	3.	92		CITA	≤ 2	2.	95
B.suis bv2	TSA	> 1	1.	REF	*B.ovis*	TSA	≤ 2	1.	REF
	MTM	≤ 0.5	3.	95		MTM	≤ 1	3.	84
	JM	b	4.	77		JM	< 1	4.	7.5
	FM	≤ 0.5	3.	58		FM	< 1	4.	8
	TM	≥ 1	2.	93		TM	≤ 1	3.	63
	CITA	≤ 0.5	3.	90		CITA	≥ 1	2.	51
B.suis bv3	TSA	≥ 1	2.	REF					
	MTM	≥ 1	2.	70					
	JM	<0.5	3.	77					
	FM	c	4.	77					
	TM	> 1	1.	83					
	CITA	> 1	1.	75					
B.suis bv4	TSA	≥ 1	1.	REF					
	MTM	≥ 1	1.	96					
	JM	≤1	2.	95					
	FM	< 1	3.	84					
	TM	≥ 1	1.	94					
	CITA	≤ 1	2.	93					

a. Colonies with ≤1mm diameter were observed on 8th day of incubation
b. Colonies with ≥1mm diameter were observed on 6th day of incubation.
c. Colonies with ≥1mm diameter were observed on 7th day of incubation.

PR results of the media for all strains except *B.ovis* in Table 6 were found to be higher than 58%. PR of JM and FM for *B.ovis* were lower than the other media's PR. The average PR results of the media for *B.suis* biovars are illustrated in Table 7. All these calculations for *B.suis* biovars were carried out based on the total colony counts on basal medium TSA, for which the average colony count was found to be 189 CFU.

Table 7 The average productivity rate of media for *B.suis* strains

Media	PR %
MTM	87.6
FM	74.2
CITA	89
JM	80.2
TM	89.6

In Table 8 below, only the statistically significant results of PR for *B.suis* biovars are listed. The statistical analysis was carried out between TM, CITA and MTM, which have the highest three PR, and JM and FM, which have the lowest PR among these five, respectively. All the results between these 3 productive media (TM, CITA, MTM) and JM and FM were found to be statistically significant.

Table 8 The statistical results of the average productivity rate of media for *B.suis* strains

Chi-Square Tests	PR	
Pearson Chi-Square	X^2 value	P value
TM &FM	14.910	.000
TM&JM	5.970	.015
CITA &FM	13.745	.000
CITA& JM	5.214	.022
MTM &FM	11.598	.001
MTM & JM	3.883	.049

There were apparent variations between the media for *B.ovis*. Particularly FM and JM showed the lowest PR. PR calculation was conducted in accordance with the total colony count (165 cfu/dish) on TSA basal medium. The statistical analysis of PR for *B.ovis* is given in Table 9.

Table 9 The statistical results of the average productivity rate of media for *B.ovis* strains

Chi-Square Tests	**PR**	
Pearson Chi-Square	X^2 value	P value
MTM&FM	190.401	.000
MTM&TM	19.122	.000
CITA&TM	1.025	.311
CITA&FM	88.088	.000
MTM &CITA	29.567	.000
FM&JM	0.040	.841

As indicated above, the difference between MTM, CITA and TM media and FM and JM reveal statistical significance. There is also significance obtained between MTM, which has the highest PR for *B.ovis* with CITA and TM.

In this study, colony growth of strains was observed during the incubation period and the findings associated with colony size and the day on which the colonies were identified is summarized in Table 10.

Table 10 The ranking of the media acc.to colony diameter for all the reference strains

Medium	**FM**	**CITA**	**MTM**	**JM**	**TM**
The strains having the highest colony diameters for each medium	*B.abortus* bv1,bv2	*B.abortus* bv5,bv6, bv9 *B.suis* bv3 *B.ovis*	*B.abortus* bv5-bv6 *B.ovis* *B.suis* bv4	*B.abortus* bv1,bv4, S19	*B.abortus* bv3,bv5, bv6, bv9 *B.suis* bv1,bv2, bv3,bv4, bv5
The strains having the lowest colony diameters for each medium	*B.abortus* bv5,bv6 *B.suis* bv1,bv3, bv4,bv5 *B.ovis*	*B.abortus* bv2,bv3, bv4	*B.abortus* bv1,bv4, bv9,S19	*B.abortus* bv2,bv3, bv6 *B.suis* bv2 *B.ovis*	*B.abortus* bv2,bv4

The most rapid colony growth of 9 strains among 14 reference strains occurred when using TM. The color of the test media is very light yellow with a transparent view like CITA and FM that makes the observation of the colonies easier. The final pH value measured for TM was about 7.2± 0.2 and gel strength of the TM including the same amount, 15 gr agar per liter, like in the FM, is similar to the standard solid medium gel strength. The slowest colony growth of 4 strains by MTM and 7 strains by FM were obtained at the end of the incubation.

Discussion

This study aimed to compare and contrast the rates of productivity belonging to the selective media by using *Brucella* strains. The PR of all media showed variety for *B.abortus* biovars particularly for *B.abortus* biovar-2. There are also statistically significant differences between average PR values of media for all *B.abortus* biovars including biovar-2. Parallel to these differences, FM was considered to provide successful isolation for *B.abortus* biovar-2, which is more fastidious among *B.abortus* biovars (Farrell, 1974). The reason behind these low PR values of media except FM might be due to the differences of medium content such as basal medium and antibiotics. The amount of polymyxin in FM is lower than that in JM and TM. Amphotericin, on the other hand, is included in all media except for FM. It is stated that the minimum inhibitory concentration (MIC) values of polymyxin and amphotericin are lower for *B.abortus* biovar-2 in comparison with other biovars (Farrell and Robertson, 1967; Farrell, 1974; Jensen and Halling, 2010). Additionally, a study by Jensen and Halling (2010) reported that *Brucella* species are more sensitive to polymyxin than colistin. MIC of colistin was found to be 2-4 times higher than polymyxin in their study. The lowest MIC (6 µg/ml) of polymyxin was identified for *B.abortus* biovar-2 and biovar-6; for this reason, they were considered to be the most sensitive biovars.

Parallel to this, Roberson *et al*. (1973), indicated that *B.abortus* biovar-2 is the most sensitive biovar against some of the therapeutic agents used in the treatment of brucellosis. In our study, the amount of amphotericin in the media is lower than the MIC value for *B.abortus* biovar-2. Although the concentration of amphotericin is low in the media, it may cause a decrease in the PR depending on the changes in the optimum growth conditions with the effects of other antimicrobial agents added to the media.

In the development of FM, amphotericin-B was not considered to be a suitable selective agent for the selective media regarding MIC values and the isolation results during the development of FM (Farrell, 1974). It was reported that the growth of *B.abortus* biovar-2 in Ryan and Mair media, which are blood agar-based media, was not observed. It was pointed out that the antibiotic combination including amphotericin and polymyxin leads to some inhibitor effects on some strains of *B.melitensis, B.suis* and *B.ovis* (Farrell and Robertson, 1972; Farrell, 1974; Murray and Corbel, 2005).

TM including erythritol showed low PR% for *B.abortus* bv2. In their study, in which they developed *Brucella* Modified Selective (BMS) medium, Her *et al.* (2010), found that *B.abortus* biovar-2 was proportionally susceptible to erythritol. The reason

behind this was the reduced recovery rates following the inoculation with delayed growth. An increase of erythritol concentration from 0.1% to 0.2% led to a significant decrease in the recovery rates from 67% to %33. Therefore, in the development of more effective selective media, checking the media through more fastidious biovars might be recommended.

Moreover, differences were identified between the productivity rates of media for *B.suis* strains. The lower rates of FM and JM than other media were also found to be statistically significant. While FM and JM include different antibiotics, the same amount of bacitracin is included in both of them. Previous studies revealed that FM poses an inhibitor effect on the growth of *B.suis* because of nalidixic acid and bacitracin (De Miguel *et al.,* 2011). JM had a higher productivity rate than FM for *B.suis*. A possible explanation for this could be the absence of nalidixic acid in JM. In the scope of isolation percentage, FM showed a lower isolation rate with 58.3% than LNIV-M and MTM for *B.suis* in a comparison study (Ferreira *et al.,* 2012). MTM which has the highest productivity rate for the majority of the strains used was also defined in the same way in a previous study comparing the selective media (Ledwaba *et al.,* 2020).

The lowest colony growth of *B.ovis* was observed in JM and FM. Additionally, the productivity rate of JM and FM for *B.ovis* was found to be 7.5% and 8%, respectively, which was significantly lower than the other media's productivity. While bacitracin concentration is lower than MIC value for *B.ovis*, it is 6 times higher than the concentration that enables growth. This finding was referred as the inhibitor effect of bacitracin in previous studies (Marin *et al.,* 1996[a]). Likewise, in a recent study; it was observed that a better amount of growth was achieved by Modified CITA than FM (Ledwaba *et al.,* 2020).

Sensitivity differences of *Brucella* species and biovars against the antibiotics that could be used either for treatment or for selective media were identified in previous studies (Farrell and Robertson, 1967; Robertson *et al.,* 1973). Robertson *et al.* (1973) used three different inoculation methods to detect the MIC of antibiotics. It was stated that the variation between the results stems from the different number of bacteria in each inoculum. The *Brucella* might be included in placenta, cotyledon and amniotic fluid of infected pregnant ruminants. The amount of *Brucella* burden in allantoic fluid and cotyledon can be up to 1×10^{10} cfu/ml and 1×10^{13} cfu/g, respectively (Yumuk and O'Callaghan, 2012; Poester *et al.,* 2013; Perez-Sancho *et al.,* 2015). These high numbers in the diagnostic materials might increase the isolation possibility with selective media demonstrating a low productivity rate.

The colony growth in 8 different reference strains amongst 14 strains occurred rapidly in TM. While it is not included in selective media except TM in this study, erythritol is one of the main components of TM, which is an effective sugar alcohol for tissue tropism of *Brucella* bacteria (Keppie *et al.,* 1965; García-Lobo and Sangari Garcia, 2005; Her *et al.,* 2010). Modified *Brucella* selective (MBS) medium was developed for the isolation of *B.abortus* strains. Erythritol was used in this medium to promote the growth of strains, which is delayed by the antibiotic component of the selective media (Her *et al.,* 2010). In the present study, it is a significant finding that TM is placed on the top in order to stimulate the colony growth for all of the *B.suis* biovars. It showed the same effect on 4 of *B.abortus* biovars. The fact that the TM medium gave the highest percentage of *B.suis* biovars in the average productivity percentage of 92.1% suggests that there may be a direct correlation between the colony growth rate of the medium and the productivity percentage. In a study, in which a medium containing erythritol was used for the isolation of *B. abortus*, it was revealed that the colonies were larger and could easily be identified 3 days after inoculation (Her *et al.,* 2010). In the light of the findings of the study, the addition of erythritol component to the selective media in order to increase the isolation sensitivity can be recommended to researchers, except for the S-19 strain.

Colony growth was slowest in 7 of the reference strains including *B.ovis* on FM and in 4 strains on MTM medium. Average productivity rate of FM for *B.suis* biovars was found to be lower than those of the other media. In addition to this, on FM, the growth of 4 biovars among *B.suis* biovars was found to be the slowest colony growth. These findings indicate that there might be a correlation between low productivity and slow colony growth as seen in the results of FM. In a comparison study of media, it was revealed that the colony size of *B.suis* was smaller in FM than MTM (Ferreira *et al.,* 2012).

In another study investigating the effect of polymyxin on the isolation of *Brucella* species, the researchers found that the relative susceptibility of reference strains to antibiotics differed widely. In this context, they reported that although the genomic sequences are very similar between species, the differences between them affect the LPS and membrane composition (Jensen and Halling, 2010). It has also been stated that the ecological range of the genus *Brucella* has expanded since the determination of the first selective medium due to the newly added species and isolated strains to the *Brucella* genus isolated in different hosts such as marine mammals (Godfroid *et al.,* 2005), baboons and catfish (Pappas, 2010; Yumuk and O'Callaghan, 2012). In this context, it is possible that the new strains and novel strains of the *Brucella* genus have different resistance or susceptibility to antibiotics. There is also an increased likelihood of exposure to atypical field strains exhibiting an atypical profile on biotyping. The effects of antibiotics in the selective medium on the growth of *Brucella* strains were evaluated in several studies (Marin *et al.*,1996[a]; Her *et al.,* 2010; Jensen and Halling, 2010; Ferreira *et al.,* 2012). Such kinds of important studies form the basis for the development of the selective media. Therefore, the antibiotics and their concentration in TM were chosen according to the present selective media content. The preferred concentrations in TM were lower than the concentrations enabling growth (CEG) revealed in previous studies (Marin *et al.,* 1996[a]; Ferreira *et al.,* 2012). In addition to this, without erythritol, the antibiotic mixture of TM was composed only of 4 antibiotics to decrease a possible cumulative inhibition effect on *Brucella* strains.

Maintaining research on the isolation and inhibition abilities of selective media will provide significant support for disease control programs. Due to the lack of specificity of serological tests, it was emphasized by the researchers that bacterial isolation in animals is indisputably the only method to prove infection (Ferreira *et al*., 2012). Therefore, the importance of bacterial isolation, in which selective media play a key role for bacteriological culture as a common method for the diagnosis of brucellosis in livestock, should not be overlooked. In a previous research, it was revealed that the isolation of causative agents of disease for control and eradication programs is very important in terms of enabling the identification of disease mediators as well as reaching the epidemiological source using molecular methods (Her *et al*., 2010).

The media in this study were observed to have adequate productivity rates for the majority of the reference strains. These productivity rates were determined by detecting the growth of reference strains in the media. Therefore, the productivity rates of the media can be considered as a guiding tool in the media preference for the samples where the number of target microorganisms is low and the contaminant load is high. The appearance time of colonies in the medium was accepted as a useful finding in the choice of medium in order to provide ease of isolation before the contaminants cover the surface of the medium. The results of the study confirmed the detectability of the reference strains conveying concrete data for the researchers for the medium preference depending on the target *Brucella* species and biovars. To conclude, the results reveal that there could not be an entirely excellent medium for the isolation of *Brucella* including several species and strains with different susceptibility and growth characteristics. Every medium may provide both advantages and disadvantages so that every medium may have the potential for improvement. Therefore, for prospective studies, determination of these potential points should be found out by checking the performance of the selective media for *Brucella* isolation and contaminant inhibition particularly by novel strains.

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