TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2017) 41: 294-301
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doi:10.3906/vet-1603-9

Detection and economic impact related to bovine respiratory disease, shrink, and traveling distance in feedlot cattle in Northwest Mexico

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Received: 03.03.2016 • **Accepted/Published Online:** 01.08.2016 • **Final Version:** 19.04.2017

Abstract: Bovine respiratory disease (BRD) is the main reason for economic loss in feedlots. A cross-sectional study was conducted in order to detect the pathogens involved in BRD. A total of 88 animals with respiratory signs were sampled for diagnosis using PCR. The detected pathogens were bovine respiratory syncytial virus (80.6%), *Mannheimia haemolytica* (79.5%), *Pasteurella multocida* (68.1%), parainfluenza 3 virus (23.8%), bovine herpes virus-1 (20.4%), and bovine viral diarrhea virus (11.3%). The average number of treatments applied per animal was 1.3 with an estimated treatment cost of 16 USD per treatment/animal. Animals receiving more than one treatment averaged costs exceeding 38 USD per animal. In total, 77% of all animals received the first treatment during the first 3 weeks after arrival. We found a relationship between shrink percentage and the distance traveled by the livestock from its origin to the feedlot (P = 0.049). BRD represents a costly condition for feedlot operations. It is imperative that cattleman and veterinary practitioners recognize the necessity to apply additional preventive medicine strategies to reduce the impact of BRD. Preconditioning and a more systematic use of methaphylaxis may improve our ability to accurately identify the most effective strategies to reduce the impact of BRD.

Key words: Bovine respiratory disease, feedlot cattle, diagnosis, shrink, treatments, costs

1. Introduction

Bovine respiratory disease (BRD) is the most common and costly disease of cattle causing approximately 75% of the morbidity and over 50% of the mortality in feedlots. BRD is a disease of the lower respiratory tract of cattle that is multifactorial in origin and results in bronchopneumonia (1). Typical viral pathogens responsible for BRD include bovine herpes virus-1 (BoHV-1), bovine viral diarrhea disease (BVDV), parainfluenza 3 virus (PI3V), and bovine respiratory syncytial virus (BRSV), while the most cited bacteria include *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* (2). BRD develops in cattle due to stress factors such as weaning, transportation, pooling of cattle from multiple sources, dusty conditions, parasitism, concurrent diseases and weather extremes (1). BRD has a major impact on the feedlot industry. Economic losses are due to mortality, costs of therapy and prophylaxis, and reduced performance. The main challenge for veterinarians and animal owners is the establishment of an accurate and timely diagnosis of ill and dying cattle to implement intervention strategies to minimize and control BRD. In the United States, the average treatment costs for BRD have been calculated as 18.00 USD per sick animal, ranging from $11.48/head of livestock treated only one time up to $37.34 for animals receiving two or more treatments (3). A recent study conducted in Veracruz, Mexico, reported up to 18.9% morbidity for BRD in feedlot cattle, representing 84.5% of all disease cases identified monthly, with an average of 15 kg net weight loss between time of arrival and application of first treatment (4). Stress factors involved in the transportation of cattle trigger a number of negative consequences, including altered nutritional status and animal behavior, reduced feed consumption and body weight (BW) gain, decreased immune function, increased morbidity and mortality due to BRD, and death. The most important of these stressors is shrink, which is the amount of BW lost during periods of feed and water deprivation and represents the reduction of BW not only from feces and urine, but also from other body tissues. Shrink can produce

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between 2% and 5% loss of live BW during transportation and can be higher when extreme conditions are present, like extreme weather during the peak of summer or winter months. In a previous work, the effects of shrink were associated with BRD morbidity and overall mortality risks (5). Approximately 340,000 heads of cattle are purchased each year and transported by trucking companies to the state of Baja California (Baja) from yearling/stocker operations located in 23 different states of Mexico. The Mexicali valley feedlots are the final destination of about 95% of the cattle introduced to Baja. Currently, the methods used to diagnose BRD in feedlot cattle include the assessment of respiratory signs such as cough, nasal discharge, increased respiratory rate or effort, and animal behavior. Although clinical signs may raise the suspicion of BRD infection, laboratory confirmation is needed to make a definitive diagnosis. BRD diagnosis can be achieved by a variety of serological methods, agent isolation, fluorescent antibodies tests, immunohistochemistry, and molecular-based tests (6). Irrespective of the infectious agent involved, the presenting clinical signs of BRD can appear similar. Moreover, the detection of bacterial pathogens can mask an underlying viral disease and virus isolation may not always be successful compared to molecular detection methods. Molecular detection of the etiological agents of BRD through polymerase chain reaction (PCR) and real-time polymerase chain reaction (RT-PCR) can provide rapid results and can detect, differentiate, and provide a quantitative result for many different targets without any single target influencing the detection of the others (7).

The aim of this study was to establish the prevalence of *M. haemolytica*, *P. multocida*, BRSV, BoHV-1, PI3V, and BVDV using PCR and RT-PCR in sick animals showing respiratory signs in a feedlot located in Mexicali, Baja California, Mexico. The economic impact related to the number of treatments applied, percentage of shrink, and effect of long-distance transportation are also estimated.

2. Materials and methods

All animal handling and sampling procedures were conducted following the guidelines of approved local official techniques for animal care, including NOM-051-ZOO-1995: Humanitarian care of animals during mobilization and NOM-024-ZOO-1995: Animal health stipulations and characteristics during transportation of animals, and NOM-033-ZOO-1995: Humanitarian care and animal protection during slaughter process. The protocols were evaluated and approved by the institutional committee for animal ethics, which is represented by the Academic Group of Animal Health and the Academic Group for Diagnosis of Infectious Diseases, both part of the Institute for Research in Veterinary Sciences, Autonomous University of Baja California, Mexicali, Baja California, Mexico.

2.1. Feedlot and animals

A cross-sectional study was conducted in a feedlot located in Mexicali, Baja California, in Northwest Mexico with an installed capacity for 10,000 heads of cattle. This feedlot was selected because the number of animals introduced is very constant throughout the year. Upon arrival, animals are sorted into groups by weight and sex. The groups are further separated into lots according to their average weight. At the feed yard, water and fodder are provided along with a 24- to 48-h period of rest. After the resting period, cattle receive a methaphylaxis protocol consisting of vaccination and a mixture of antibiotics and antiparasitic drugs. If the BW of cattle is above 300 kg, the animals are sent directly to the fattening process with high energy rations from 90 to 120 days. If the initial weight of livestock is below 300 kg, cattle are sent to pasture fields until they gain the required weight to initiate the fattening process. After the feedlot, when animals weigh 500 to 550 kg, they are sent to slaughter.

2.2. Sample size

The size of the required sample for disease detection was calculated using a formula (8). For this calculation, the following values were considered: a population of 10,000 animals, 6% prevalence (2), 99% confidence level, and 95% sensitivity. The size of the sample considered for disease detection was 79, although 9 more samples were analyzed.

2.2.1. Sample collection

During routine inspections at the reception feedlot or before methaphylaxis application, those animals classified as clinically ill by the site veterinarians were used for random selection. For this purpose, a list of random numbers for the selection of animals was used. A total of 88 whole blood and nasal swab samples were collected from randomly selected animals showing fever, labored breathing, cough, nasal discharge, diarrhea, loss of appetite, and reluctance to move.

2.3. DNA and RNA extraction

Nucleic acid was extracted using the DNeasy Blood & Tissue Kit and RNeasy Mini Kit (QIAGEN, Valencia, CA, USA). Nasal swabs were vortex-mixed at maximum speed for 30 s and the swab was removed from tube and centrifuged at 5000 × *g* for 2 min to pellet bacteria for DNA extraction. Next, 250 µL of transport media supernatant was used for viral DNA and RNA extraction and 20 µL of white blood cells from buffy resuspended in 230 µL of 0.9% NaCl solution was used for extraction of BVDV RNA. Extracted DNA and RNA were quantified and stored at –80 °C until molecular diagnosis testing.

2.4. Primers

The *Mannheimia haemolytica* and *Pasteurella multocida* PCR primers were sourced from a previous study (9). The real-time PCR primers were designed using the NCBI/

Primer-BLAST Primer Designing Tool based on highly conserved regions of viral genomes where there was sufficient data to be confident of the consensus sequences. The characteristics of the primers are outlined in Table 1.

2.5. PCR protocols

End-point PCR assay was conducted in a reaction volume of 25 µL for the detection of *Pasteurella multocida* and *Mannheimia haemolytica*. Mixtures and PCR conditions were performed according to the mentioned study (9) adding 2.5 µL of PCR 10X enhancer solution (Invitrogen, Waltham, MA, USA). *M. haemolytica* and *P. multocida* DNA-positive controls were obtained from bovine lung isolates, kindly provided by Dr Jose Barajas, Department of Microbiology, Faculty of Veterinary Medicine, National Autonomous University of Mexico.

Real-time PCR reactions were run in a CFX96 Real-Time PCR thermal cycler (Bio-Rad, Hercules, CA, USA) using a standard reaction mixture containing 100 ng of sample DNA, one set of primers, Bio-Rad iQ SYBR Green SuperMix for DNA samples or iScript One-Step RT-PCR master mix kit with SYBR Green for RNA samples, plus sterile deionized water for a total volume of 25 µL. Positive DNA or RNA controls for BVDV, BRSV, PI3V, and BoHV-1 were extracted from a commercial live-modified virus vaccine (Bovimune Protector 5 Diamond Animal Health, Des Moines, IA, USA). RT-PCR for DNA samples was performed with an initial incubation period of 10 min at 95 °C followed by 40 cycles of 20 s at 95 °C for denaturation and 45 s at 60 °C for annealing and extension. For RNA samples, reverse transcription for DNA synthesis was performed at 50 °C for 10 min and inactivation of the reverse transcriptase enzyme at 95 °C for 3 min, followed by 40 cycles at 95 °C for 20 s for denaturation and 45 s at 60 °C for annealing and extension. A melt-curve analysis from 65 °C to 95 °C was used in all RT-PCR reactions to identify primer-dimers or nonspecific amplified PCR products. RT-PCR data were downloaded in 96-well plate format from Bio-Rad CFX Manager 2.1 to MS Excel and analyzed manually. All PCR and RT-PCR samples were analyzed in duplicate, including nontemplate and sterile deionized water controls.

2.6. Cost estimation of treatments

The cost of each individual treatment per animal was calculated by multiplying the number of days that the animal was receiving treatment by the average cost of the treatment used (10).

2.7. Association of shrink and place of origin of cattle

Shrink is expressed as the percentage of change in BW of cattle before and after shipment from their places of origin. Total cattle weight per trailer was obtained in their place of origin and upon arrival to the feedlot in Baja California immediately after unloading and before the animals had access to water or food. This information was obtained from the databases of the feedlot. A questionnaire was applied to collect data that included from which state of Mexico the cattle were purchased, number of days traveling to Baja, number of days since arrival, average BW at place of origin and arrival, clinical history, presumptive diagnosis, and vaccinations and treatments received.

2.8. Statistical methods

Linear regression analysis was performed using the Statistix 9 Analytical Software (Tallahassee, FL, USA) to establish the association between transportation distance and shrink percentage. For analysis, $P < 0.05$ was considered

Table 1. Nucleotide sequences of primers used in PCR and RT-PCR.

Pathogen		Sequence 5′-3′	Gene	Fragment length
Pasteurella multocida	Forward	AGG TGA AAG AGG TTA TG	*Omp87*	219
	Reverse	TAC CTA ACT CAA CCA AC	*Omp87*	219
Mannheimia haemolytica	Forward	TTC ACA TCT TCA TCC TC	*ssa*	325
	Reverse	TTT TCA TCC TCT TCG TC	*ssa*	325
BVDV	Forward	GGTAGTCGTCAGTGGTTCGAC	*5'-UTR*	89
	Reverse	CGTCCAGATTAGGATGTGCTG	*5'-UTR*	89
BRSV	Forward	GCAATGCTGCAGGACTAGGT	*N protein*	85
	Reverse	GCATATGCTTTGGCAGCATC	*N protein*	85
PI3V	Forward	GGAAGATGGGCAGAATGTACTC	*M matrix protein*	114
	Reverse	CAGTTGCGTTGACGTGGA	*M matrix protein*	114
BoHV-1	Forward	GTGAACTGCATCGTGGAAGA	*UL27*	80
	Reverse	ATAATGTCCCCGGTCGAGAG	*UL27*	80

The primers were used at a concentration of 400 nM.

significant. The relative frequency of each of the bacterial and viral diseases included in this study was estimated by dividing the number of animals diagnosed as positive for a given test by the number of analyzed animals (11).

3. Results

3.1. Detection of the pathogens involved in BRD

3.1.1. PCR

The PCR showing identification of *P. multocida* and *M. haemolytica* was confirmed by conventional PCR. Of the 88 obtained samples, 70 were positive for *M. haemolytica* and 60 for *P. multocida.*

3.1.2. RT-PCR

Samples were considered as positive when a sigmoidal amplification curve was displayed similarly to the positive control before cycle 35. During the assays, a positive amplification was observed in positive controls and in 10 samples for BVDV, 71 samples for BRSV, 18 samples for BoHV-1, and 21 samples for PI3V. Our study found that 48 out of 88 animals (54%) tested positive for three or more pathogens. Furthermore, three animals tested negative for all pathogens.

3.2. Pathogen distribution across the Mexican Republic states

Samples comprising 88 bovines were obtained from 11 states in the republic. The most frequently detected pathogens were BRSV (80.6%), *M. haemolytica* (79.5%), and *P. multocida* (68.1%). The pathogen distribution according to origin is presented in Table 2. It should be noted that *M. haemolytica* and *P. multocida* were detected in most of the states, whereas BVDV was identified in fewer states.

3.3. Treatment frequency in sick animals and costs

The frequency of treatments for BRD per animal was variable. This resulted in 1.3 average treatments per animal. From all of the analyzed bovines, 43 animals received a first treatment and 18 received a second, while 6 animals received a third treatment (Table 3). There was even 1 animal that was positive for *P. multocida* and BRSV that received 4 treatments.

The associated economic losses are summarized in Table 3. The treatment protocol for BRD comprises the application of florfenicol, enrofloxacin, ceftiofur, and penicillin G procaine as the first, second, third, and fourth treatments, respectively. The average treatment cost per animal was 16 USD for those receiving the first treatment, 6 dollars for the second treatment, and 8 dollars for a third or fourth treatment. The animals that received more than one treatment incurred costs of up to 38 USD per animal.

3.3.1. Number of days from arrival to first BRD treatment

The average number of days after which the animals received a first treatment against BRD was 17. It was also observed that 77% of the animals received a first treatment during the first 3 weeks after their arrival, as shown in Figure 1.

3.4. Shrink percentage regarding origin

The average shrink of the analyzed animals was 13.31%, ranging from 7.4% to 16.03%. Figure 2 shows the shrink percentage according origin per federal entity. The distance traveled between the site where animals were bought and the feedlot varied between 803 and 3412 km, with an average of 2381.6 km. In order to perform a linear regression analysis, we included 87.5% of the shipments,

Table 2. Distribution of pathogens involved in BRD corresponding to all analyzed samples.

States	*M. haemolytica*	*P. multocida*	BVDV	BRSV	BoHV-1	PI3V
Chiapas	0	1	0	1	0	0
Chihuahua	16	17	5	18	6	8
Guerrero	16	11	4	21	1	6
Jalisco	14	12	0	9	3	1
Nuevo Leon	7	8	1	8	4	2
Oaxaca	2	1	0	1	1	0
Sinaloa	3	1	0	3	0	0
Sonora	2	2	0	0	1	0
Tamaulipas	5	3	0	7	1	2
Veracruz	3	3	0	1	1	2
Zacatecas	2	1	0	2	0	0
Total (%)	70 (79.5)	60 (68.1)	10 (11.3)	71 (80.6)	18 (20.4)	21 (23.8)

BVDV, Bovine viral diarrhea virus; BRSV, bovine respiratory syncytial virus; BoHV-1, bovine herpesvirus-1; PI3V, parainfluenza type 3 virus.

Table 3. Treatments and costs related to BRD.

Number of antibiotic treatments	Antibiotic	Medical expenses, $/animal	Number of animals
0	--	0	20
1	Florfenicol	16	43
2	Enrofloxacin	6	18
3	Ceftiofur	8	6
4	Penicillin G procaine	8	1
Total		38	88

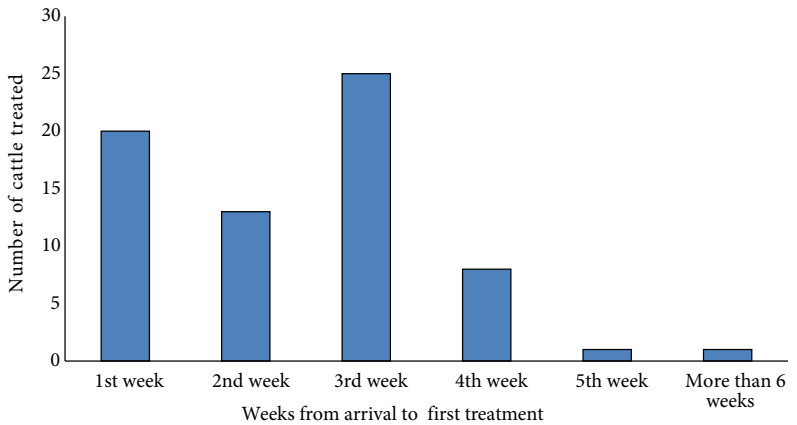

Figure 1. Distribution of the first treatment for BRD after entering the herd.

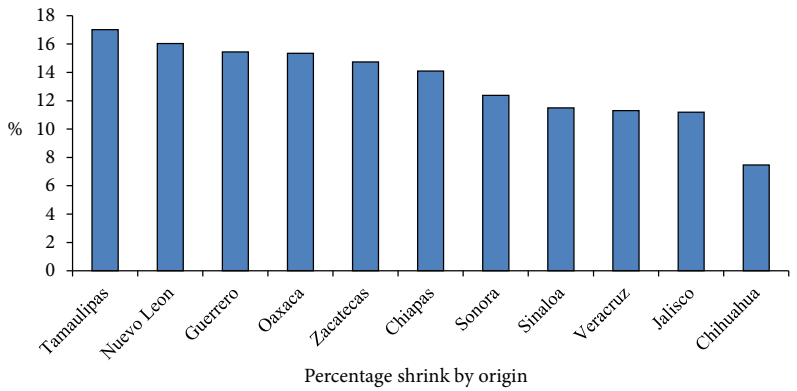

Figure 2. Shrink percentage according to the site of origin.

from which the greatest percentage of the samples were obtained. We identified a linear relationship between distance and shrink percentage. The correlation coefficient between the latter variables was 0.77 (P = 0.049). Thus, for each additional 500 km of distance, there was additional shrink of 4.24%.

4. Discussion

The present study found different pathogens involved in BRD using PCR. According to the data obtained with regard to the frequency of pathogens, our results differ from those of other authors (12,13). They found a prevalence of *P. multocida* from 7.11% to 14.09% in the states of Hidalgo and Queretaro, as well as a prevalence of *M. haemolytica* of 28.03%, which might have been caused by the use of bacterial culture as a diagnostic technique. PCR, on the other hand, is a more sensitive test that allowed the detection of a number of cases that failed to be detected by culture (6). Studies carried out in Colima and Yucatán found the BRSV average seroprevalences to be

50.8% and 90.8%, respectively (14,15), which are similar to the results found in this study. Other studies carried out in Mexico found a prevalence of 54.4% for BoHV-1 and 85.6% for PI3V (14,16). In our study, 18% and 21% of the animals tested positive for BoHV-1 and PI3V, respectively. In the case of BVDV other authors found a prevalence of 14%, whereas our study found that 11.3% of the analyzed samples were positive. Differences in prevalence between studies could be explained by aspects such as regions, diagnostic techniques, production systems, herd size, and handling practices (17).

Regarding the possibility of some animals presenting more than one pathogen, our study found that 48 out of 88 animals tested positive for three or more pathogens using PCR. These results are similar to those obtained by previous works (18), which demonstrated that simultaneous infections with several pathogens are very frequent. Other researchers found a relationship between several aetiological agents and the severity of BRD. In the pathogenesis of BRD, viruses play a key role, harming the respiratory tract and facilitating secondary infections (19). When BVDV is present, for instance, the severity of *M. haemolytica* infection is increased (18).

A decrease in weight equal to 4.9%–7.9% was found, as well as a relationship between the distance animals are transported and the percentage of the expected shrink (20). Our study found an average shrink of 13.31%, giving a positive relationship between the distance cattle travel from their origin and the percentage of shrink.

Previous studies estimated the treatment costs and obtained a range from $11.09 to $18 (3,21). These results are similar to those obtained in our study.

In the studied feedlot, the animals received the first treatment for BRD within an average of 17 days after landing. Our data show that 77% of the animals received treatment during the first 21 days, indicating that after arriving at the production unit, BRD cases increased during this period and then declined. This decrease in BRD cases for newly arrived animals might be related to the incubation period, which varies from 7 to 30 days (22). The temporal distribution in days for the first BRD treatment differed from other studies in which the animals usually received the first treatment after 30 days (23). Additionally, other studies pointed out that 81% of the first treatments occurred within the first 42 days (21). It has been found that the peak BRD incidence occurred 18 days after the arrival of the animals to the farm, and 87% of the first treatments were given during the first 35 days (24). These variations might be caused by several different factors (transportation, handling, origin of animals, production system) that occurred in the herds in which the studies were carried out.

A previous study showed that the average number of treatments for bovines infected with BRD was 1.6 per animal (21). In our study, there was a similar average (1.3 treatments). Even if the cost of treatments to deal with BRD is substantial, the impact of BRD on the production of beef cattle can be even more significant. It is estimated that 21% of the total losses attributed to BRD are treatment-related costs. The remaining 79% is attributed to the decreased weight in the carcass (8.4% lower) and the quality of meat (25); even those animals that were treated once, twice, or more times showed a decrease in the price of carcass equal to $23, $30, and $54, respectively (26). Similar studies are necessary in our region in order to estimate total losses (mortality, treatments, weight of carcass, and quality of meat).

One limitation of our study was that the RT-PCR test does not allow us to differentiate between a vaccine strain and a field strain; it is clear that some of the detected cases might have been false positives due to the effect of the vaccine strain (27,28). Nevertheless, considering that the vaccine strain is not always detected (29,30), all animals showed signs and typical symptoms of respiratory deficiencies, and some animals had not been vaccinated at the time of sampling, so it is possible that those cases were experiencing the disease. On the other hand, three animals tested negative for all pathogens. This is probably due to the presence of microorganisms associated with BRD that were not considered in this project (*H. somni* and *M. bovis*) and might explain why these animals were negative for all tests, despite showing clinical signs.

Due to the great quantity of livestock that is brought into the state and the few zoosanitary requirements, it is possible that some of the animals that are brought in suffer from different diseases in different stages, which are triggered on farms soon after arrival. For this reason, a diagnostic system that is fast and precise is necessary; this might help to quickly introduce improved handling procedures. Our study showed, through molecular diagnosis, the pathogens involved with BRD in the beef cattle production systems, which appeared frequently. It was observed that BRD causes an economic impact, due to the high cost of treatments, which can increase if considering additional veterinary care and the extra days that sick animals spend on the farm. On the other hand, there is a relationship between the percentage of shrink and the distance traveled by cattle from their origin to the farm, significantly affecting the producer's profit margin.

Due to the features of the present study, the results cannot be extrapolated to the total population of cattle introduced annually to feedlots in Baja California; nevertheless, it is clear that there are BRD issues in animals that cause considerable losses. In order to estimate the economic impact of these diseases on production systems, it is necessary to design epidemiological studies with a larger number of samples that allow estimates of frequency, distribution, and risk factors associated with the

occurrence of these diseases in the state of Baja California, including the tracking of these production animals to evaluate the negative effects on the carcass features and in order to quantify the economic losses caused by BRD.

Acknowledgments

This work is part of the requisites to obtain the grade of Doctor en Ciencias Agropecuarias of the present first author (Universidad Autónoma de Baja California). We thank Fernanda Bermudez, Tomás Cardenas, Jaqueline Moreno, Nadia Ruiz, Javier Palacios, Héctor Moisés Rangel Pantoja, and Carolina Trillo for their excellent technical assistance. This work was supported partly by the Convocatoria 2012 del Programa de Mejoramiento del Profesorado, Fortalecimiento de los Cuerpos Académicos (Secretaría de Educación Pública).

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